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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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Interaction of Autonomic Drugs on Isolated Mouse Intestine*

By LLOYD W. HAZLETON† and EMILY GODFREY†

The isolated mouse intestine as a tool for studying the actions of autonomic drugs was investigated in some detail. A comparatively simple method for studying the action of these drugs in the lumen, or with the lumen excluded, is described. In general, the mouse intestine is comparable to other isolated intestines in its reaction to these drugs. Of the drugs studied, only acetylcholine was totally inactive when confined to the lumen and none was destroyed in the lumen.

THE PHARMACOLOGY of autonomic drugs such as acetylcholine, atropine, and physostigmine was studied as early as 1906 and 1907 by Hunt and Taveau (1), Unger (2), and Magnus (3). The latter two investigated the action of the drugs on the mammalian intestines *in situ*, using the cat. Alvarez (4) in 1918 used five isolated segments of rabbit's intestines suspended in the same bath to show the varied reactions of seventy-five drugs on different parts of the intestine. Bernheim (5) in 1934 was among the first to study carefully the white mouse, using isolated small intestine.

Bernheim (5) studied the interaction of acetylcholine and adrenaline on the isolated small intestine and colon of practically all laboratory animals including mouse, rat, guinea pig, rabbit, fish, cat, and dog. Gunn (6) in his recent report on the antagonism of the two drugs used isolated rabbit intestine. To contrast the technique used by each of these workers is interesting because of the individual approach to the identical problem. Bernheim administered the drugs separately, which is the usual method, while Gunn mixed the drugs in varying concentrations, and both workers proved physiological antagonism between acetylcholine and adrenaline. A preliminary report by Hazleton and Godfrey (7) described a method for studying the action of autonomic drugs placed in the lumen of mouse intestine and presented some of the more fundamental results. Somewhat later Meyer and McEwen (8) used a modified Trendlenburg method to study the action of drugs on the serosa and mucosa of isolated rabbit and guinea-pig intestine in an attempt to differentiate the finer responses of the intestinal muscles.

The objective of the following report is to present the results of additional studies employing the mouse method and to describe some of the characteristic interactions of the various autonomic drugs.

* Received Feb. 24, 1948, from the Kalusowski Memorial Research Laboratory, School of Pharmacy, The George Washington University, Washington, D. C. Acknowledgment is made of a grant from the Proprietary Association in support of this study.

† Present address, Hazleton Laboratories, Falls Church, Va.

APPARATUS AND TECHNIQUE

Tubular segments of small intestine were suspended between a fixed and a freely moving hollow glass cannula as illustrated in Fig. 1. These were placed in a smooth-muscle chamber contained in a constant temperature tank at 37.5°. Fluid or air was used consistently for placing drugs into the lumen. The open end of the moving cannula extended sufficiently above the fluid level of the chamber to permit maximum contraction of the muscle without immersing the cannula. Since the upper end of each cannula was open to the air, the fluid pressure in the lumen was at equilibrium with the

TABLE I.—EXPLORATORY TESTS TO DETERMINE THE EFFECTIVE DOSAGE RANGE AND INTERACTION OF ONE OR MORE DRUGS WHEN PLACED IN THE BATH[a]

Drug	Conc.	Vol./ 100 Cc.	Followed by	Conc.	Vol./ 100 Cc.	Results
Acetylcholine	1:10,000	1.0	.....	....	...	Maximum typical contraction
	1:10,000	0.02	.....	....	...	Standard dose giving uniform contraction[b]
	1:10,000	0.01	.....	.....	...	Minimum contraction
	1:10,000	0.02	Atropine	1:1,000	0.01 0.02	Relaxation
	1:10,000	0.05	Epinephrine	1:1,000	0.05	Drop in base line and cessation of activity for a short time
Atropine	1:1,000	0.01	.....	....	...	Relaxation with level base line
	1:10,000	0.01	.....	....	...	Relaxation with level base line
	1:100,000	0.01	.....	....	...	Relaxation with level base line
	1:1,000	0.01	Acetylcholine	1:10,000	0.01	No acetylcholine contraction
	1:1,000	0.01	Acetylcholine	1:10,000	0.02	After repeated washings, normal response
	1:1,000	0.02	Acetylcholine	1:10,000	1.0	Acetylcholine response equal to that of standard dose
Physostigmine	1:1,000	0.1	.....	....	...	Paralysis
	1:10,000	0.1	.....	....	...	Paralysis
	1:100,000	0.1	.....	....	...	Paralysis
	1:1,000,000	0.1	.....	....	...	Paralysis
	1:10,000,000	0.1	.....	....	...	Enhancement of activity
	1:10,000,000	0.1	Acetylcholine	1:10,000	0.005	Response comparable to the minimum typical contraction of acetylcholine
Prostigmine	1:4,000	0.1	.....	....	...	Paralysis
	1:8,000	0.1	.....	....	...	Paralysis
After several washings of bath			.....	....	...	Enhancement and prolongation of acetylcholine activity
	1:4,000	0.1	Acetylcholine	1:10,000	0.01	More uniform and prolonged reaction than physostigmine. Contraction comparable to 0.02 of acetylcholine
Epinephrine	1:1,000	0.01	.....	....	...	Drop in base line, temporary decrease of activity
	1:10,000	0.01	.....	....	...	Drop in base line, temporary decrease of activity
	1:100,000	0.01	.....	....	...	Drop in base line, temporary decrease of activity
	1:10,000	0.01	Acetylcholine	1:10,000	0.05	Response comparable to standard dose of acetylcholine when epinephrine was not present

[a] Except as noted, the bath was not washed prior to addition of the second drug.
[b] The standard dose designates that dose which produced a fairly uniform but submaximal response in satisfactory muscles.

could be placed in the lumen by means of a tuberculin syringe joined to the top of the fixed cannula by a short rubber tube. The cannulae used in these experiments required a volume of 0.4 cc. to fill them and the intestinal segment to a level approximating the fluid level of the chamber. This volume external pressure. The chamber contained oxygenated Locke-Ringer's solution while the drugs were dissolved in distilled water. When drugs were placed in the lumen there thus existed an osmotic gradient favoring interchange between the lumen and the chamber. Freedom of experimental design

was somewhat limited by the fact that the only means of removing drugs from the lumen was to expel them into the chamber, with air or fluid from the syringe.

Segments of ileum 3 to 4 cm. in length were obtained immediately after sacrificing mice of either sex by cervical dislocation. The segments were freed of mesentery and fecal material, suspended as described above, filled with Locke-Ringer's solution and allowed to relax for the normal tracing. Muscles which did not exhibit some degree of spontaneous activity were rejected as unsatisfactory.

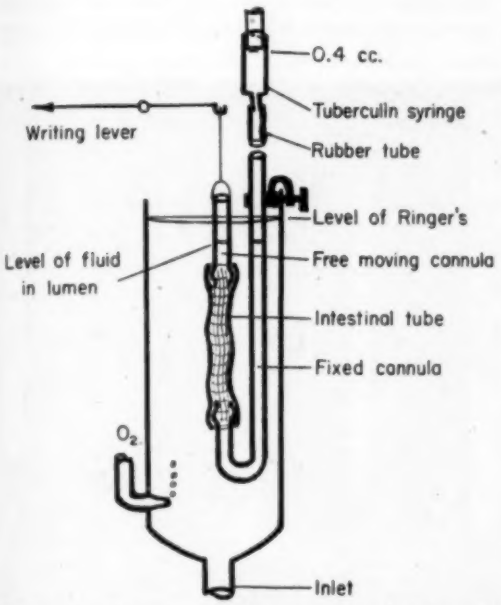

Fig. 1.—Diagram of apparatus for studying the effect of drugs in the lumen of the isolated mouse intestine. In practice two muscles were placed in each bath, one serving as a control.

To avoid thermal shock all solutions were maintained at 37.5° by immersing the containers in the constant temperature tank. Under these conditions the segments remained active over periods of several hours.

Two separate muscles were usually suspended in each chamber, the first being the test segment and the second, the control. The control provided a simultaneous normal response for comparison with the treated muscle response under the various conditions of the experiment. In addition it served to detect the inadvertent contamination of the chamber by the drugs.

RESULTS

The results to be presented here are largely confined to the methodology and the more obvious action mechanisms. To avoid excessive discussion these will be presented by a series of figures and tables with individual consideration of the illustrative points.

Table I presents the results of exploratory tests to determine the effective doses and interactions of drugs placed in the chamber. As might be expected, these results correspond well with those obtained in the usual smooth-muscle experiments in other animals where the exterior and lumen both contact the solution. No evidence was obtained that exclusion of the lumen significantly altered the results. In all but extremely small doses the muscles were paralyzed by physostigmine and prostigmine, and it was found that best enhancement of acetylcholine activity was obtained after these drugs had been removed from the chamber. The usual atropine-acetylcholine antagonism was observed, but it is interesting to note that following removal of atropine from the chamber the standard dose of acetylcholine was ineffective while (*a*) large doses of acetylcholine overcame the residual atropine effect and (*b*) repeated washings restored the effectiveness of the standard dose.

Table II combines several phases of the problem by selecting the more classical actions and interac-

TABLE II.—ACTION AND INTERACTION OF AUTONOMIC DRUGS UNDER VARIOUS CONDITIONS

Action of Drug in Lumen of Test Muscle, Volume—0.4 Cc.				Interaction of Acetylcholine (1: 10,000 conc.) When Placed in the Bath While the First Drug is Present in the Lumen of the Test Muscle			After Washing the Bath of All Acetylcholine, Action of Both Muscles on Expelling the Drug from Lumen of the Test Muscle	
Drug	Conc.	Test Muscle	Control Muscle	Quantity of Acetylcholine, Cc.	Test Muscle	Control Muscle	Test Muscle	Control Muscle
Acetylcholine	1:10,000	N	N	....	..	..	+	+
Atropine	1:1,000	–	N	0.01–1.0	–	+	–	–
	1:10,000	–	N	0.01–1.0	–	+	–	–
	1:100,000	–	N	0.01	N	+	–	–
	1:100,000	–	N	1.0	+	+	–	–
Physostigmine	1:1,000	–	N	0.01–1.0	–	+	–	–
	1:10,000	–	N	0.01–1.0	–	+	–	–
	1:100,000	–	N	0.01–1.0	–	+	N	N
	1:1,000,000	N	N	0.005	+	+	N	N
	1:10,000,000	N	N	0.005	+	+	N	N
Prostigmine	1:4,000	–	N	0.005–1.0	–	+	N	N
	1:8,000	–	N	0.005	+	+	N	N
Epinephrine	1:1,000	–	N	0.02	+	+	–	–
	1:10,000	–	N	0.02	+	+	–	–
	1:100,000	N	N	0.02	+	+	–	–

N—normal activity.
+—contraction typical of drug involved.
–—relaxation and/or cessation of normal activity which was of varying periods of duration.

tions of autonomic drugs. Several of these and a few which do not lend themselves to the tabulation deserve consideration.

The first portion of the table summarizes activity of drugs confined to the lumen of one intestinal tube and indicates that there is no interchange of drugs between the lumen and the surrounding bath since in no instance was the control muscle affected.

The center portion of the table is concerned with the interaction between lumen-contained drugs and acetylcholine. In general, this confirms the noninterchange of drugs since the control muscle responds to acetylcholine within normal limits. In the test muscle a typical atropine-acetylcholine antagonism is obtained throughout the normal range of doses, as illustrated in Fig. 2. By varying the ratio of doses, however, a gradation of antagonism is obtained. Physostigmine and prostigmine exhibit antagonistic and synergistic properties comparable to those obtained with these drugs in the bath. It was also observed that in addition to sensitizing the action, the prolongation of acetylcholine response was marked following the smaller (nonparalytic) doses of these drugs.

The interaction between lumen-contained epinephrine and acetylcholine is perhaps the most interesting of the series. Even very active muscle strips were completely relaxed by epinephrine in the lumen but responded in normal manner following the addition of acetylcholine to the chamber. This is illustrated in Fig. 3. The ability of epinephrine to produce complete relaxation without material interference with the acetylcholine reaction is in sharp contrast to the action of atropine under similar circumstances. In the very active muscles atropine reduces spontaneous activity but in so doing completely blocks the normal acetylcholine response. It was further observed that following the explusion of epinephrine (1 to 1000) from the lumen and washing the epinephrine from the bath the administration of acetylcholine into the lumen produced a very strong contraction. Although the explanation for this phenomenon is not readily apparent, it is the only circumstance under which an acetylcholine response was elicited by the drug in the lumen.

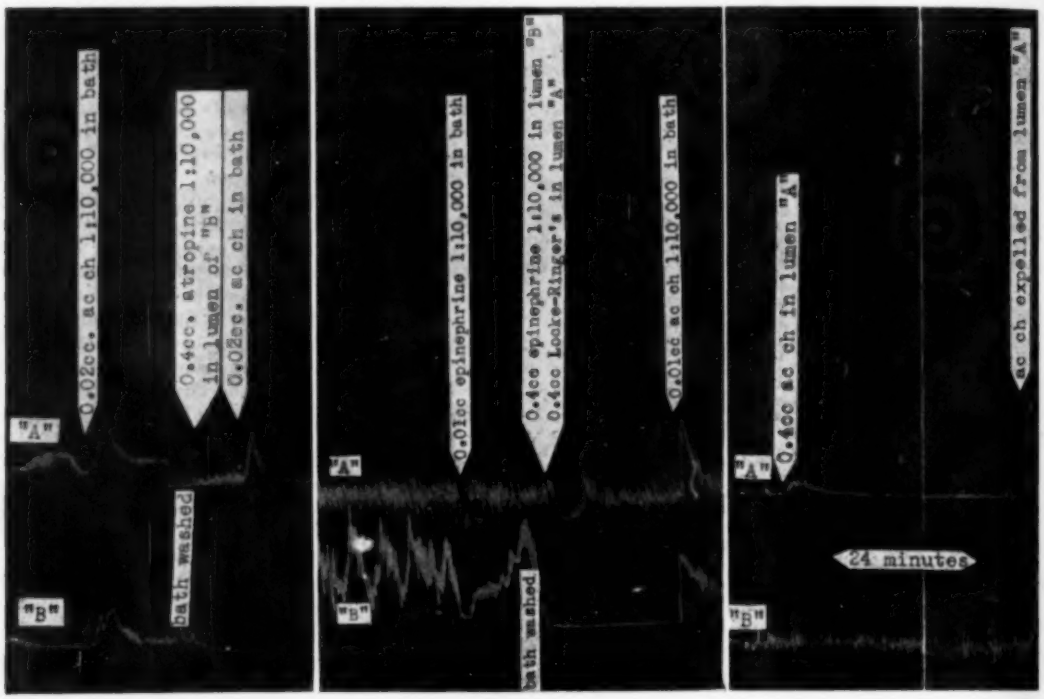

Fig. 2 Fig. 3 Fig. 4

Fig. 2.—Showing action of atropine in the lumen, noninterchange between lumen and bath, and antagonism of acetylcholine in bath.

Fig. 3.—Showing action of small dose of epinephrine in the bath, prolonged relaxation from epinephrine in the lumen, and absence of antagonism to small dose of acetylcholine in the bath.

Fig. 4.—Demonstrating the ineffectiveness and stability of acetylcholine in the lumen, and the function of a control muscle.

The third portion of Table II summarizes the responses obtained following expulsion of the lumen-contained drugs into the bath. The actions obtained under these conditions, particularly those in the control muscle, indicate that none of the drugs studied was inactivated by confinement within the lumen (Fig. 4). Under these conditions the most dilute solutions of physostigmine produce an increase in activity which is not antagonized by normal doses of atropine. It is to be assumed that this is due to the continued activity of residual drug in the lumen of such intensity that externally applied atropine is incapable of complete antagonism.

SUMMARY

In general, the response of the mouse small intestine to various classical autonomic drugs may be considered to be analogous to those of other mammals. The convenience, cost, and availability of this investigatory tool would seem to merit more widespread usage than it has enjoyed in the past.

By use of a simplified technique for the study of action of drugs in the lumen it appears that several interesting fields of more detailed investigation have been revealed. The method is such that it eliminates many of the complicated features of those previously used for this approach to the problem, and while it is subject to further improvement it appears to be reliable for at least fundamental observations. Not the least of the desirable features is the use of a continuous control muscle, making possible the avoidance of inadvertent errors and the evaluation of the various responses under controlled conditions. It is to be hoped that this report will stimulate interest both in the mouse intestine as a tool and in the method which has been applied.

REFERENCES

(1) Hunt, R., and Taveau, R. DeM., *Brit. Med. J.*, **1906**, 1788–91.
(2) Unger, M., *Arch. ges. Physiol.* (Pflüger), **119**, 373–403 (1907).
(3) Magnus, R., *ibid.*, **102**, 123, 349(1904).
(4) Alvarez, W. C., *J. Pharmacol. Exptl. Therap.*, **11**, 171 (1918).
(5) Bernheim, F., *ibid.*, **51**, 59(1934).
(6) Gunn, J. A., *Quart. J. Pharm. Pharmacol.*, **18**, 108–16 (1945).
(7) Hazleton, L. W. and Godfrey, E., *Federation Proc.*, **4**, 124(1945).
(8) Meyer, A. E. and McEwen, J. P., *Am. J. Physiol.*, **148**, 715–25(1947).

Spectrophotometric Determination of Procaine in Procaine Penicillin G*

By C. V. ST. JOHN†

In a search for a rapid method for the determination of procaine in procaine penicillin G a spectrophotometric method was developed. A series of lots analyzed by the spectrophotometric method and by the chloroform extraction-titrimetric method shows comparable results. Measurements were made on a Beckman spectrophotometer at 290 mμ.

DURING the experimental stages of the development of procaine penicillin (3) it was necessary to determine the procaine content of the final procaine penicillin G to determine whether or not excess procaine had been carried along with the precipitate. Since additional procaine greatly increases the toxicity of the product this determination was of great imporance.

The conventional method (2) for the determination of procaine involves the addition of an excess of ammonium hydroxide, extraction of the procaine base with chloroform, evaporation of the chloroform, addition of a known amount of hydrochloric acid, and back titration of the excess with sodium hydroxide. Since this involves much time a spectrophotometric study of the absorption spectra of procaine was made. It was found that the peak of the absorption band for procaine exists at 290 mμ.

EXPERIMENTAL

Apparatus and Solution.—Transmittancy measurements were made with a Beckman quartz spectrophotometer (1) equipped with 1.000-cm. quartz cells and an ultraviolet radiation source operating with a spectral band width of approximately 1–5 mμ. Distilled water was used in the reference cell.

A standard solution of procaine hydrochloride was made from a lot of material that analyzed 100.0% by the chloroform shake-out procedure. This solution was freshly prepared whenever used.

Effect of Interferences.—A certain amount of interference is to be expected in any method of this

* Received May 7, 1948, from Antibiotics Manufacturing and Development Dept., Eli Lilly and Company, Indianapolis, Ind.

† The author is indebted to D. E. Flick for analyzing the series of lots of procaine penicillin by chloroform extraction-titrimetric method.

type. It is understood that any compounds that absorb ultraviolet radiation at 290 mμ will interfere. Penicillin G does not interfere at the concentration equivalent to the amount of procaine being measured. Acid decomposition of the equivalent amount of penicillin G gives some interference as shown in Fig. 1; however, in this laboratory no interference has been encountered in the final product. If interference exists some method of removing or correcting for this interference must be developed.

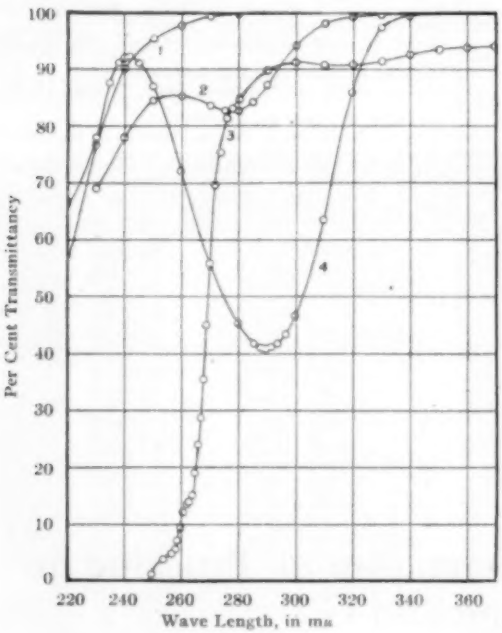

Fig. 1

1. Approximately 0.008 mg. potassium penicillin per ml.
2. Approximately 0.008 mg. potassium penicillin per ml. decomposed with HCl and heat.
3. Approximately 2.0 mg. potassium penicillin per ml.
4. 0.005 mg. procaine base per ml.

Conformity to Beer's Law.—The system conforms to Beer's law at concentrations within the range of the instrument.

Effect of *p*H.—The *p*H range of the crystalline product (5.0–7.5) as specified by the Federal Food and Drug Administration does not significantly change the *p*H of the final dilution. It is interesting to note that on all the material produced in our laboratories the *p*H of the crystalline product has been between 5.7 and 6.1.

Effect of Concentration.—The lower limit of procaine concentration which can be determined by this method is fixed by the maximum cell length and interferences; the upper limit is fixed by the error encountered by repeated dilution. The concentration range of the final dilution for a 1-cm. cell is 0.001–0.010 mg. of procaine base per ml.

Comparison of Spectrophotometric and Chloroform Extraction-Titrimetric Method.—A series of final lots of procaine penicillin was analyzed by both methods by separate operators. The results are shown in Table I. The results obtained show very little deviation; however, care must be taken to clean the cells frequently with dichromate-sulfuric acid cleaning solution and to make careful adjustment of the instrument. Cell calibration should also be checked. The arithmetical average of the readings for several adjustments of the instrument on each of two fillings of the cell should be taken. If care is exercised results comparable to those shown in Table I can be expected.

TABLE I.—COMPARISON OF SPECTROPHOTOMETRIC METHOD WITH CHLOROFORM EXTRACTION-TITRIMETRIC METHOD

Lot No.	Chloroform Extraction Method, % Procaine	Spectrophotometric Method, % Procaine	Deviation, %
1	40.0	39.9	−0.25
2	39.7	39.7	0.00
3	39.8	39.9	+0.25
4	39.8	39.9	+0.25
5	40.3	40.2	−0.25
6	40.2	39.9	−0.75
7	39.7	40.0	+0.75
8	39.8	40.3	+1.25
9	39.8	40.2	+1.00
10	40.4	40.3	−0.25
		Average Deviation	±0.5

Recommended Procedure

Weigh accurately approximately 50 mg. of procaine penicillin G and transfer to a 100-ml. volumetric flask. Dilute to volume with distilled water and shake until completely dissolved. Transfer 5 ml. of this dilution to a 250-ml. volumetric flask, dilute to volume, and measure the optical density of the resulting solution at 290 mμ against water in the reference cell. Obtain the concentration of the solution by calculation from an extinction coefficient determined on a sample of pure procaine hydrochloride or a sample of pure procaine penicillin G analyzed by the chloroform shake-out titrimetric procedure.

SUMMARY

A spectrophotometric method for the determination of procaine in procaine penicillin G has been described. The accuracy of the method compares very favorably with the conventional method, especially when care is exercised in dilution, cleaning of absorption cells, and in the adjustment of the instrument. Interference has not been encountered in the analysis of the final product. The procedure is simple and requires less time than the conventional method.

REFERENCES

(1) Cary, H. H., and Beckman, A. O., *J. Optical Soc. Am.*, **31**, 682(1941).
(2) "National Formulary," Eighth Edition, Mack Printing Company, Easton, Pa., 1946, p. 416.
(3) Sullivan, N. P., Miller, H. C., and Rhodehamel, H. W., Jr., *Science*, **107**, 169(1948).

Pharmaceutical Applications of Isopropyl Alcohol. III. Solubilities of Sedatives, Hypnotics, and Sulfonamides*

By HENRY M. BURLAGE†

In a previous paper (1) the solubilities of local anesthetics in isopropyl alcohol have been reported. The present work is a continuation of the determinations of this constant and is applied to official and nonofficial sedatives, hypnotics and sulfonamides. The procedure employed is outlined therein (1). The results are shown in Tables I and II.

TABLE I.—SOLUBILITIES OF SEDATIVES AND HYPNOTICS IN ISOPROPYL ALCOHOL

Name of Compound	Manufacturer	Solubility, Gm./100 Cc. of Solution
Barbituric Acid Derivatives		
Alurate N. N. R.	Hoffman-LaRoche	30.5430
Sodium Alurate N. N. R.	Hoffman-LaRoche	25.5750 (d.)
Sodium Amytal	Lilly	32.1730
Barbital U. S. P.	Merck	11.5772
Veronal N. N. R.	Winthrop	11.2520
Barbital Sodium	Merck	0.3008
Veronal-Sodium N. N. R.	Winthrop	0.3710
Dial N. N. R.	Ciba	8.2990
Ipral Calcium N. N. R. (Probarbital)	Squibb	0.2920
Ipral Sodium N. N. R.	Squibb	0.8750
Neonal N. N. R.	Abbott	53.0670
Ortal Sodium N. N. R.	Parke Davis	27.9500
Pentobarbital Sodium	Gane's	2.5010
Pentothal Sodium N. N. R.	Abbott	19.8370 (d.)
Phenobarbital U. S. P. (Phenobarbitone)	Gane and Ingram	11.7012
	Merck	11.6128
Luminal N. N. R.	Winthrop	11.3480
Phenobarbital Sodium	Gane and Ingram	14.7910
Luminal Sodium N. N. R.	Winthrop	14.5950
Sandoptal N. N. R.	Sandoz	30.2900
Seconal Sodium N. N. R.	Lilly	60.7620
Vinbarbital Sodium N. N. R.		
Delvinal Sodium	Sharp and Dohme	9.4900 (d.)
Compounds Containing Bromine		
Bromural N. N. R.	Bilhuber Knoll	9.7560
Carbromal N. F.	Merck	7.1620 (d.)
Adalin	Winthrop	6.4360
Hydantoin Derivatives		
Diphenylhydantoin Sodium U. S. P.		
Dilantin Sodium	Parke Davis	4.8810
Sulfonmethanes		
Sulfonethylmethane	Merck	11.2170
Sulfonmethane N. F.	Merck	2.1290

(d.) denotes decomposition.

TABLE II.—SOLUBILITIES OF SULFONAMIDES IN ISOPROPYL ALCOHOL

Name of Compound	Manufacturer	Solubility, Gm./100 Cc. of Solution
Sulfonamide Compounds		
Sulfadiazine U. S. P.	Squibb	0.0430
Sulfaguanidine U. S. P.	Squibb	0.1770
Sulfamerazine U. S. P.	Sharp and Dohme	0.1740
Sulfanilamide U. S. P.	Gane and Ingram	0.7970
Sulfapyrazine N. N. R.	Mead Johnson	0.0290
Sulfapyridine U. S. P.	Merck	0.1750
Sulfathiazole U. S. P.	Merck	0.5750
Succinylsulfathiazole U. S. P.		
Sulfasuxidine N. N. R.	Sharp and Dohme	0.5690
Sulfamyd	Schering Corp.	5.6650
Sulfonamide Sodium Salts		
Sulfadiazine Sodium U. S. P.	Squibb	0.1860
Sodium Sulfamyd	Schering Corp.	0.6290
Sulfapyrazine Sodium N. N. R.	Mead Johnson	0.7080

SUMMARY

The solubilities, in isopropyl alcohol, of 28 sedatives and hypnotics and of nine sulfonamide compounds and three sulfonamide sodium salts, on the American market, are reported on the basis of weight in volume.

REFERENCE

(1) THIS JOURNAL, 36, 16(1947).

* Acknowledgment is made to Enjay Company, Inc., of New York for funds to carry on this study and to the various manufacturers who generously furnished the supplies of the sedatives and hypnotics used.

† Professor of Pharmacy, University of North Carolina School of Pharmacy, Chapel Hill, N. C.; now Dean, College of Pharmacy, University of Texas, Austin, Texas.

Effect of Pruning and Exfloration on the Growth and Alkaloidal Content of *Datura metel* Linn.*

By S. PRASAD†

Pruning and exfloration are known to affect vegetative and reproductive vigor in plants. While much work has been carried out during recent years on a variety of economic plants, no such study has been made of medicinal plants. The present investigation deals with the effect of pruning and exfloration on the growth and alkaloid formation in *Datura metel*.

PRUNING and exfloration are known to affect the vegetative and reproductive vigor in plants. A good deal of work has been carried out during recent years on a variety of economic plants (1–10). Severe pruning, in general, produced inhibitory effects and reduced the yield; mild operation, on the contrary, increased the yield. Removal of flower stalks and axillary buds, in case of tobacco, resulted in an increase of the size of the leaves (13), and in tomatoes defruiting or exfloration greatly increased the size of the treated plants (5, 6). In soy beans, Austin (11) noted no such stimulating effect.

Barring crop plants and fruit trees, little attention has so far been paid to investigating the effects of pruning and exfloration on medicinal plants. While studying the effect of mineral deficiency on *Datura alba*[1] (14), it was discovered that maximum alkaloid content was formed at the time of flowering and thereafter the alkaloidal content decreased. It was, therefore, decided to ascertain if the yield of the leaves and the alkaloidal content could be increased by pruning and/or suppressing the flower bud formation. Observations recorded in these directions are presented in this contribution.

EXPERIMENTAL

Healthy seeds of *Datura metel* were selected with respect to their uniformity in size and weight and germinated in well-prepared seed beds. A month later when seedlings were of 3–4 cm. in height, they were transplanted in test plots 15 × 18 ft. in size; 42 plants were sown in each plot at a distance of $2^3/_4$ ft. each way. Treatments, seven in number, were replicated four times. Each block thus contained seven randomized plots, receiving the following treatments:

1. Control (no treatment given).
2. Most of the branches pruned (heavy pruning).
3. Only one branch from the base pruned (mild pruning).
4. Flower buds removed from only one branch, *viz.*, the main half stem from the base.
5. Flower buds removed from the whole plant (early exfloration).
6. Flower buds removed from the whole plant at a later stage (late exfloration).
7. Flower buds removed from the whole plant early and also treated a month before exfloration with ammonium sulfate (20.6% N) at the rate of 600 Gm. per plot.

After one and a half months when the seedlings were established fully in the plots and the plants bore a few branches, pruning was done. Care was taken that no injury to the main stem was caused. Exfloration was done on some plants at an early stage and on others at later stages when the flower buds were formed. All effects were compared against the control which consisted of unpruned and unexflorated plants.

The procedure adopted for measuring plant characters and for estimating alkaloids was the same as employed before (14) for this plant species.

RESULTS

General Growth Characters.—The control plants showed their normal growth and development and formed flowers when they were four months old; later at intervals of twelve to fourteen days new crops of flowers were formed which bore fruits. Plants from which most of the branches were removed (treatment no. 2) were severely injured and were not able to produce new shoots until fairly late in the season; it was about a month and a half after pruning that new shoots developed on the main stem, more commonly at the base of the pruned branches. Treatment No. 3, where only one branch from the base was removed, showed profuse growth and compared favorably with the control. Pruning failed to increase the growth of these plants; flowering was also delayed in these pruned plants.

Delayed flowering was a distinct feature in all exflorated plants. In treatment no. 5, where flower buds were continuously removed, new flower buds developed a week later than those of the normal plants. Even under treatment no. 4, where only one branch from the base was exflorated, delayed flowering was noted by four to five days; while nonexflorated branches on such plants exhibited intense blossoming, the exflorated ones on the same plant showed only the presence of buds at a well-developed stage. In plants where flower buds were removed late (treatment no. 6), flowering was delayed by two to three days. In treatment no. 7, where early exfloration was preceded by treatment

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† Assistant Professor of Pharmacognosy, Department of Pharmaceutics, Benares Hindu University, India; now a Watumull Fellow at the School of Pharmacy, University of Wisconsin, Madison, Wis.

[1] According to H. A. Timmerman, *Datura alba* Nees is *Datura metel* Linn. (*Pharm. J.*, 1927, pp. 571–574). Hence to maintain the priority, *D. metel* Linn. has been used in this contribution, for *D. alba* Nees, the latter name having been employed by the writer in his previous publications.

with nitrogenous fertilizer, production of flowers was delayed by about a week.

Vegetative growth was very much increased in all the exflorated plants. Height and thickness of stem and number and size of the leaves and the branches were increased over those of the normal plants.

Stem Elongation.—Height in severely pruned plants (treatment no. 2) was significantly decreased at all stages in the life cycle, in response to heavy pruning (Table I). In mildly pruned plants, pruning affected height adversely in the beginning; at later stages, however, height approached more or less the control. In exflorated plants, height of plants was significantly increased at the majority of the stages. Greatest increase in stem elongation was noted in exflorated plants treated with nitrogen (treatment no. 7). Treatment nos. 5, 4, and 6 followed next in order.

Leaf Number.—Number of leaves was significantly reduced in pruned plants. When pruned mildly, reduction was not significant due to formation of new branches and leaves. In exflorated plants, number of leaves increased at all stages; greatest increase was noted in early exflorated and nitrogen-treated plants. Next in order of increase were the plants of treatment nos. 5, 6, and 4.

Dry Weight of Plants.—Dry matter of entire plant of treated and control sets was likewise affected in the order mentioned above. Heavily pruned plants (treatment no. 2) were greatly affected; dry weight production was significantly reduced at practically all the stages of the life cycle. Mildly pruned plants

TABLE I.—AVERAGE PLANT CHARACTERS OF DATURA METEL, AS AFFECTED BY EXFLORATION AND PRUNING

	Treatment Numbers							
Age, Mo.	1 Control	2 Heavy Pruning	3 Mild Pruning	4 Exfloration in Half Stem	5 Early Exfloration	6 Late Exfloration	7 Early Exfloration and Treated with Ammonium Sulfate	S. E.[c]
				Stem Height in Inches				
2	4.85	.....	.....	.....	.....	.....	.....	.....
3	8.60	7.40	7.85	.....	.....	.....	.....	.....
4	19.85	10.55[a]	16.20[a]	22.25[b]	23.60[a]	20.10	25.05[a]	±1.024
5	26.75	16.25[a]	25.25	34.08[b]	41.13[a]	30.13	46.50[a]	±2.817
6	42.20	29.10[b]	40.85	52.60[b]	58.60[a]	52.80[b]	60.20[a]	±5.220
				Leaf Number				
2	12.5	.....	.....	.....	.....	.....	.....	.....
3	25.5	6.0	10.8	.....	.....	.....	.....	.....
4	45.0	10.5[a]	28.5[a]	50.0[a]	55.5[a]	45.00	62.00[a]	±0.850
5	85.4	20.0[a]	20.8[a]	110.0[a]	128.0[a]	104.35[a]	140.5[a]	±4.247
6	98.8	35.0[a]	68.4	220.0[a]	250.5[a]	238.50[a]	300.0[a]	±16.983
				Dry Weight of Entire Plant in Gm.				
2	1.399	.....	.....	.....	.....	.....	.....	.....
3	16.072	5.389	9.076	.....	.....	.....	.....	.....
4	39.525	10.215[a]	35.953	44.363[b]	54.250[a]	42.159	49.491[a]	±2.256
5	80.203	32.165[a]	76.593	90.297[a]	113.508[a]	94.290[a]	115.535[a]	±3.385
6	162.164	49.240[a]	152.300	189.680[a]	241.259[a]	226.258[a]	288.540[a]	±7.803
				Dry Weight of Leaf in Gm.				
2	1.104	.....	.....	.....	.....	.....	.....	.....
3	9.452	2.074	4.568	.....	.....	.....	.....	.....
4	22.280	3.240[a]	20.465	24.505	30.056[a]	23.105	35.520[a]	±2.048
5	35.700	10.603[a]	39.375	40.732[b]	50.825[a]	41.035[b]	53.075[a]	±2.429
6	68.873	20.450[a]	63.165	83.810[a]	103.103[a]	96.823[a]	124.568[a]	±3.102
				Dry Weight of Stem in Gm.				
2	0.210	.....	.....	.....	.....	.....	.....	.....
3	5.215	2.105	3.158	.....	.....	.....	.....	.....
4	14.150	4.250[a]	11.580	16.500	20.348[a]	15.804	23.846[a]	±1.462
5	36.868	17.095[a]	29.773[a]	41.470[b]	52.543[a]	41.978[b]	52.973[a]	±1.820
6	72.728	23.073[a]	65.533[a]	84.998[a]	108.120[a]	102.943[a]	133.130[a]	±2.221
				Dry Weight of Root in Gm.				
2	0.085	.....	.....	.....	.....	.....	.....	.....
3	1.405	1.210	1.350	.....	.....	.....	.....	.....
4	3.105	2.725	2.908	3.358	3.846	3.250	4.125[b]	±0.423
5	7.635	4.668[a]	7.445	8.095	10.140[b]	11.278[a]	9.848[b]	±0.857
6	20.563	5.718[a]	23.603	20.870	30.018[a]	26.593[b]	30.843[a]	±3.012
				Percentage Alkaloidal Content				
4½	0.2250	0.2045	0.2168	0.3225[a]	0.3856[a]	0.3425[a]	0.4025[a]	±0.0180
5½	0.2026	0.1920	0.1956	0.3410[a]	0.3824[a]	0.3500[a]	0.3850[a]	±0.0195

[a] Difference when compared with the control significant at 1%.
[b] Difference when compared with the control significant at 5%.
[c] Standard error of difference of means.

were affected only in the beginning (fourth month). Exflorated plants significantly increased in dry weight at all stages. Treatment no. 6, however, where exfloration was performed later, failed to increase significantly dry matter in the fourth month. Greatest dry matter production took place in treatment no. 7; treatment nos. 5, 6, and 4 followed in order.

Dry weight of component organs was affected in more or less the same order. Heavily pruned plants (treatment no. 2) showed significant reduction in leaf and stem weight at all stages (Table I); mildy pruned ones (treatment no. 3) exhibited significant reduction in stem weight only at fifth and sixth months and very little effect in their leaf weight. Increase in dry weight of exflorated plants was significant in case of treatment nos. 5 and 7 at all stages. In nos. 4 and 6, where partial or later exflorations were tried, significant effect was observed only at later periods, namely the fifth and sixth months of their life cycle. On dry weight of roots, partial exfloration or mild pruning had practically no effect; severe pruning, however, lowered dry matter and exfloration increased it significantly.

Alkaloidal Content.—Pruning of *Datura metel* did not affect the alkaloidal content of the leaves. Exfloration, early or late, on the other hand, brought about an increase (Table I.) This is in keeping with the fact that at the time of flowering, the alkaloidal content is maximum, but with the formation of the fruits and seeds a gradual decrease takes place, apparently due to the translocation of alkaloids from leaves to flowers and thence to fruits and seeds (Cf. Farr and Wright, 1904; Mueller, 1914). Exfloration checks such translocation; the alkaloidal content of the leaves, in consequence, remains at a high level throughout.

TABLE II.—THE EFFECT OF EXFLORATION IN TREATMENT NUMBER 4 (HALF EXFLORATED PLANTS)

Plant Characters[a]	Fourth Month			Fifth Month			Sixth Month		
	Exflorated	Nonexflorated	S. E.	Exflorated	Nonexflorated	S. E.	Exflorated	Nonexflorated	S. E.[d]
Shoot length, in.	16.40	14.85	±1.050	27.55[b]	22.20	±2.055	45.50[b]	38.50	±2.500
Leaf No.	29.50[c]	20.50	±1.550	69.09[c]	50.80	±3.500	130.50[c]	89.50	±5.250
Dry weight of stem, Gm.	8.450[b]	6.058	±1.005	20.350[b]	16.450	±1.450	38.256[c]	31.409	±2.056
Dry weight of leaf, Gm.	13.203[c]	11.302	±0.596	22.527[c]	18.205	±1.245	45.700[c]	38.010	±2.120
Alkaloidal content, %	0.3305	0.3205	±0.0058	0.3450	0.3360	±0.0060			

[a] Only mean values are given. The length and dry weight of the basal part of the stem common to the exflorated and nonexflorated branch have been excluded.
[b] Significant at 5%.
[c] Significant at 1%.
[d] S. E. refers to the standard error of mean.

DISCUSSION

From the foregoing it appears that pruning of *Datura metel* has no beneficial effect. On the contrary it impairs the vigor of the whole plant to such an extent that the plant is unable to compensate even at later stages. Mild pruning, however, stimulates growth, but does not lead to any increase over the control plants. Exfloration, on the other hand, brings about an all-round beneficial effect on the whole plant. Stimulating effects of exfloration on the vegetative growth and alkaloidal content are marked even in partially exflorated plants—exflorated branch significantly increases in height, leaf number and dry weight of stem and leaf as compared to the nonexflorated branch of the same plant (Table II). On alkaloid content also, exfloration tends to bring about significant increases.

Results obtained by other investigators (12, 6–10), point out that structural features of the plants in part also determine the relationship between limits of vegetative and reproductive growth. Thus in plants of determinate habit, the inflorescence utilizes all the apical meristem and hence arrests elongation. In plants of indeterminate growth habit, on the other hand, stem elongation continues until the developing fruits begin to utilize the available food supply. In the latter case again, wider differences are noted from species to species. Thus where fruiting takes place once for all in the life cycle, as in crop plants of indeterminate habit, fruiting interferes with the stem elongation. In cases like *Datura metel* where fruit setting continues for a long time, stem elongation and formation of new shoot continue for some time more even after the fruits have formed; this is the case in tomatoes as well. If exfloration is practiced on such plants vegetative growth will continue indefinitely, as shown by the results obtained in this investigation.

In tomatoes, Murneek (5, 6) observed that fruit production limits growth and exfloration or defruiting increases the size of the plant. In soybean, a short-day plant of indeterminate habit, no increase in vegetative development, following exfloration, was noted by Austin (11). Such a differential behavior of the soybean is attributed to its photoperiodic properties. In this case, shortening of day length, not only initiates the reproductive phase, but also curtails its vegetative processes. *Datura metel*, though not exactly a long-day plant, has been found to show greater vegetative growth with longer exposures of light than with shorter duration of illumination (15). Its behavior is, therefore, like that of tomatoes which when exflorated continue vegetative development indefinitely.

SUMMARY

The effect of pruning and exfloration on the growth and alkaloidal content of *Datura metel* has been described. The results indicate that heavy pruning brings about a severe injury to the whole plant and impairs its vigor so much that the plant

is not able to make good this loss until late in the life cycle. Stem height, leaf number, and dry weight are significantly depressed. Mild pruning does not affect the plant so adversely, and the pruned plants approach the control ones in their vegetative growth.

Exfloration brings about an all-round increase in these directions. Height, leaf number, and dry matter production are all significantly increased. This beneficial effect is marked even on partly exflorated plants.

On alkaloid content exfloration shows a significant effect. Pruning has no effect in this regard. Greatest yield in dry matter and alkaloid content is obtained in plants which are early exflorated and also treated with nitrogen before exfloration. Plants exflorated later or those that are partially exflorated are not benefited much in these regards.

REFERENCES

(1) Childs, W. H., *Proc. Am. Soc. Hort. Sci.*, **35**, 489–91, 1937(1938).
(2) Woods, J. J., *Sci. Agr.*, **18**, 620(1938).
(3) Thompson, H. C., *N. Y. (Cornell) Agric. Expt. Sta. Bull.*, **580**, 1(1934).
(4) Murneek, A. E., *Proc. Am. Soc. Hort. Sci.*, **22**, 196 (1926).
(5) Murneek, A. E., *Plant. Physiol.*, **1**, 3(1926).
(6) Murneek, A. E., *Missouri Agr. Expt. Sta. Res. Bull.*, **90**(1926).
(7) Gustafson, F. G., *Plant Physiol.*, **2**, 153(1927).
(8) Gustafson, F. G. and Laing, H. E., *Papers Mich. Acad. Sci.*, **13**, 95(1931).
(9) Chandler, W. H., *N. Y. (Cornell) Agr. Expt. Sta. Bull.*, 416(1923).
(10) Chandler, W. H. and Heinicke, A. J., *Proc. Am. Soc. Hort. Sci.*, **22**, 74(1926).
(11) Austin, S., *Plant Physiol.*, **10**, 225(1935).
(12) Eaton, F. M., *J. Agr. Research.*, **42**, 447(1931).
(13) Wolf, Fredrick A. and Gross, P. M., *Bull. Torrey Botan. Club*, **64**, 117(1937).
(14) Prasad, S., *Indian. J. Pharm.*, **5**, 65(1943).
(15) Prasad, S., (unpublished work).
(16) Farr, E. H. and Wright, R., *Pharm. J.*, **72**, 185(1904)
(17) Mueller, A., *Arch. Pharm.*, **252**, 280(1914).

Observations on the Leaves of Several Species of Viburnum*

By HEBER W. YOUNGKEN†

The results of a comparative study of the leaves of *Viburnum prunifolium*, *V. rufidulum*, and *V. cassinoides* are reported. Determinations of the vein islet numbers, palisade ratios and stomatal indexes of the three species are included.

IN A PAPER dealing with the history, botany, and pharmacognosy of four species of *Viburnum* (1), the author discussed the characteristics of the plants and barks of *Viburnum prunifolium*, *V. rufidulum*, *V. cassinoides*, and *V. nudum*. At that time the medicinal properties of these Viburnums were only known to occur in the bark. Since that time extracts of the leaves of several species have been reported (2) to possess properties similar to that of the barks. If further research confirms this report, the leaves may be found more economical sources of the Viburnum drugs, and their collection instead of the bark tends to conserve the natural stands of these *Viburnum* species which have undergone depletion for many years. The purpose of this paper is to report the writer's more detailed observations made on the leaves of *Viburnum prunifolium* L., *Viburnum rufidulum* Raf., and *Viburnum cassinoides* L.

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† Professor of Pharmacognosy and Biology, Massachusetts College of Pharmacy, Boston.

MATERIALS

The leaves of the *Viburnum* species described in this paper were gathered from authenticated plants growing in the Arnold Arboretum and Medicinal Plant Garden of the Massachusetts College of Pharmacy at Jamaica Plain, Mass., during 1943–1947 and from herbarium sheets and preserved specimens of leaves gathered by the writer in North Carolina and Tennessee during his collecting trips in the South previous to World War II.

PHYSICAL CHARACTERISTICS OF LEAVES

Viburnum prunifolium.—Leaves petiolate, 2.5 to 7 cm. in length and 1.2 to 3 cm., occasionally up to 5 cm. in width; lamina ovate, oval, or suborbicular, its apex rounded or acute, its base cuneate or rounded, its margin serrulate to occasionally serrate in places, and with incurved, callous-tipped teeth; upper surface dark green, sometimes tinged and spotted with purple and red, glabrous; lower surface pale green and glabrous or with rusty hairs along the midrib and in the axils of the secondary

veins; venation pinnate-reticulate; texture subcoriaceous to coriaceous at maturity; petiole short, slender, grooved above, green to purplish, 0.2 to 1.6 cm. in length, occasionally narrow winged, especially on strong shoots. As shown by Sargent (3) and also observed by the writer, the amount and nature of the pubescence on these leaves varies. In the northern United States the leaves are usually glabrous, while in the southern United States the under surface is frequently marked by the presence of numerous rusty hairs. Odor, valerian-like, on crushing or moistening; taste astringent and bitter.

Viburnum rufidulum.—Leaves petiolate, 4 to 10 cm. in length and up to 6 cm. in width; lamina ovate, elliptic to elliptic-obovate, usually obtuse but sometimes rounded or emarginate at apex, serrulate or denticulate along the margin, the teeth callous-tipped and frequently only slightly incurved, rounded, cuneate, or inequilateral at base; upper surface dark green, lustrous, and glabrous; lower surface pale green, rusty pubescent toward the base; venation pinnate-reticulate; texture coriaceous; petiole narrowly to broadly winged, 6 to 12 mm. in length, rusty-pubescent, especially beneath; odor valerian-like on crushing or moistening; taste astringent and bitter.

Viburnum cassinoides.—Leaves petiolate, the lamina elliptic, ovate, ovate-elliptic, ovate-lanciolate or oblong, occasionally oblanceolate, 3 to 10 cm. in length, and up to 4.5 cm. in width, the apex abruptly acuminate or obtuse, the base cuneate or rounded, the margin undulate or crenate to obscurely dentate. The upper surface dull green, nearly glabrous, the lower surface pale green, more or less scurfy on the veins; petioles 1 to 2 cm. in length; odor valerian like; taste astringent and bitter.

HISTOLOGY OF LEAVES

Viburnum prunifolium.—Transverse sections of the lamina outside of the midrib exhibited dorsiventral structure with an upper epidermis of tangentially elongated epidermal cells possessing a moderately thickened cuticle, a palisade parenchyma of a single layer of cells occupying about one-third of the width of the section, spongy parenchyma traversed by fibrovascular tissue, and a lower epidermis similar to that of the upper epidermis except for a somewhat thinner cuticle and the presence of stomata.

Cross sections of the midrib were convex above and below and showed an epidermis with capitate and stellate hairs. In the center occurred a meristele with narrow collateral fibrovascular bundles separated by medullary rays 1 cell in width. A pericycle surrounded the bundles which, excepting in young leaves, possessed pericyclic fibers arranged in the form of an arc together with few isolated fibers below the meristele. The region between the epidermis and pericycle was occupied by collenchyma and parenchyma, some of the cells of which contained rosette aggregates of calcium oxalate. The secondary veins were devoid of pericyclic fibers.

Surface sections of the lamina showed an upper epidermis with epidermal cells having slightly curvilinear vertical walls, and no stomata and a lower epidermis with epidermal cells having pronounced curvilinear vertical walls. Numerous oval to broadly oval stomata occurred in this epidermis. No regularity occurred in the arrangement of the subsidiary cells around the guard cells of the stomata. Frequently 5 to 6 occurred and, occasionally, one or two elongated crescent-shaped subsidiary cells were found parallel to the guard cells.

Both epidermises possessed small capitate hairs and stalked stellate hairs. The lower epidermis of young leaves showed short, 1- to 2-celled, simple nonglandular hairs, the latter with thick wall and narrow lumen.

Transverse sections of the petiole exhibited three vascular strands imbedded in parenchyma, the latter surrounded by collenchyma, scattered cells in the parenchyma containing rosette aggregates of calcium oxalate. An epidermis bearing capitate and stellate hairs covered the exterior. Some of the epidermal cells contained anthocyanin.

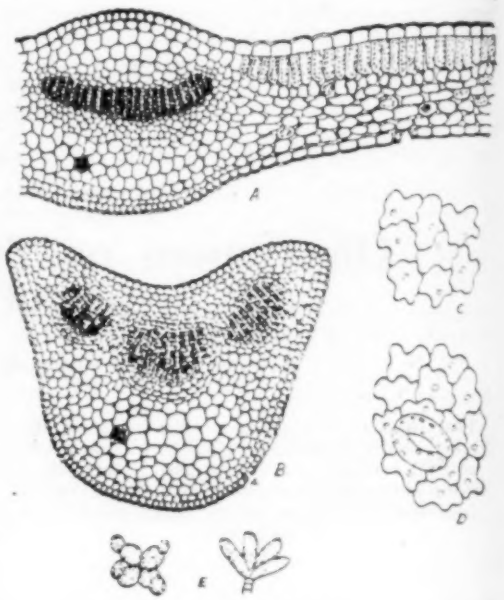
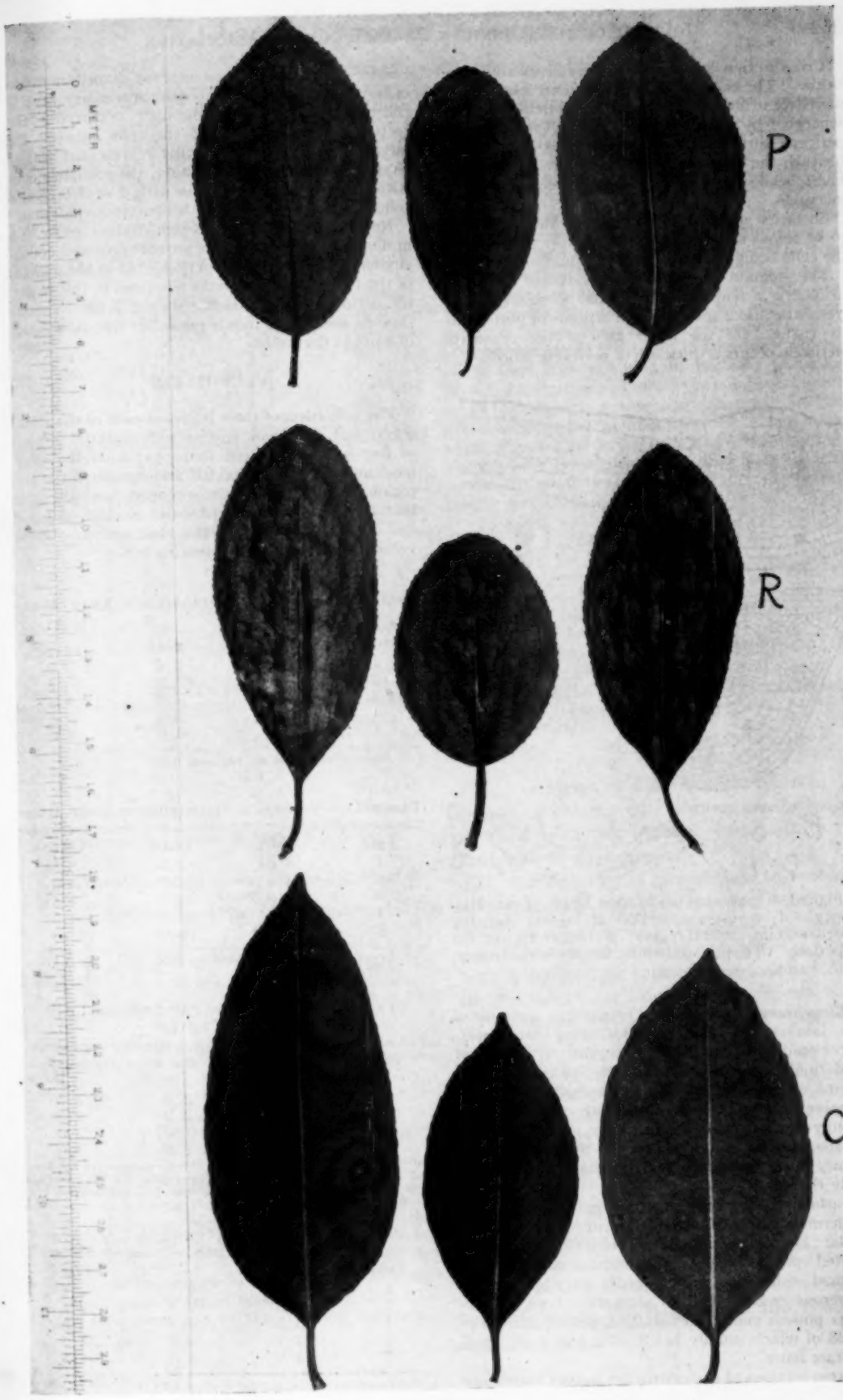


Fig. 2.—*Viburnum prunifolium* Linné. Leaf Histology. *A*, transverse section of lamina showing a secondary vein and interneural region; *B*, transverse section of petiole; *C*, upper epidermis; *D*, lower epidermis; *E*, hairs.

Viburnium rufidulum.—Transverse sections of the lamina outside of the midrib exhibit dorsiventral structure. The upper epidermis possesses tangentially elongated epidermal cells with a thick cuticle. The palisade parenchyma consists of a single layer of richly chlorophylloid cells occupying about one-fourth of the depth of the mesophyll. The spongy parenchyma consists of irregularly lobed cells and is traversed by fibrovascular tissue. The lower epidermis shows many stomata and its cuticle is somewhat thinner than that of the upper epidermis. Both epidermises show stellate hairs with short and long stalks and short-stalked peltate hairs, especially numerous on young leaves.

FIG. 1.—Viburnum Leaves. P, *Viburnum prunifolium* Linné; R, *Viburnum rufidulum* Rafinesque; C, *Viburnum cassinoides* Linné. X7/8


P
R
C

Cross sections of the midrib are convex above and below. The central region shows a crescent-shaped meristele of narrow collateral fibrovascular bundles separated by medullary rays one cell in width. A pericycle surrounds the bundles which, in the region beneath the meristele, contain an arc of pericyclic fibers, while a few pericyclic fibers occur above the meristele.

Surface sections of the lamina show the epidermises to be somewhat similar to those of *V. prunifolium*, the cells being curvilinear in outline.

The stomata of mature leaves studied were 41.8 to 53.2 μ in length. The petiole possesses a large crescentic and 2 small circular strands of fibrovascular tissue. On the lower face of the crescentic meristele occurs a thick arc of pericyclic fibers.

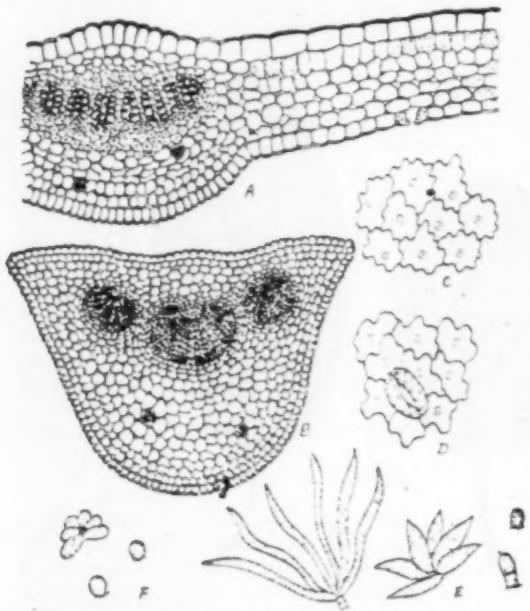


Fig. 3.—*Viburnum rufidulum* Raf. Leaf Histology. *A*, transverse section of lamina showing midrib and interneural region; *B*, transverse section of petiole; *C*, upper epidermis; *D*, lower epidermis; *E*, *F*, hairs.

Viburnium cassinoides.—Transverse sections of the lamina exhibited the following structures: upper epidermis of a layer of tangentially elongated epidermal cells with thick cuticle, palisade parenchyma of a single layer of chlorophylloid cells occupying about two-fifths the depth of the section, spongy parenchyma of irregularly lobed cells and air-spaces, the region traversed by fibrovascular tissue, lower epidermis with stomata and moderately thickened cuticle.

Surface sections of the upper epidermis showed epidermal cells with moderately curvilinear vertical walls. Surface sections of the lower epidermis exhibited epidermal cells with a striated cuticle whose vertical walls were pronouncedly curvilinear, and numerous oval to elliptic stomata. Both epidermises possess many short-stalked, peltate hairs, the shield of which usually has 8 cells, and some small capitate hairs.

Cross sections of the midrib are convex above and below. In the center is a crescent-shaped meristele of narrow bundles separated by medullary rays 1-cell in width. No pericyclic fibers occurred in the sections studied. Most of the cells between the meristele and lower epidermis represented collenchyma, while the region above the meristele consisted largely of parenchyma with 2 or 3 layers of collenchyma directly beneath the upper epidermis.

Rosette aggregates of calcium oxalate up to 39 μ in diameter occurred in the parenchyma and collenchyma of the midrib and petiole. As in the petioles of the two other Viburnums discussed in this paper, the petiole of *V. cassinoides* showed 3 fibrovascular strands and its epidermis possessed the same kinds of hairs as the lamina.

VEIN ISLETS

The vein islets of three leaves of each of the aforementioned *Viburnum* species were counted in each of five fields in cleared strips cut from the apex, base, and between the midrib and margin in the mid region of each leaf (margin-center), and the vein islet numbers ascertained by the method of Zufall and Burlage (4) with slight modification. These values are shown in the following tables.

TABLE I.—VIBURNUM PRUNIFOLIUM LEAF APEX[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	3.5	3.5	3.5
2	4.0	3.5	3.5
3	3.5	4.0	4.0
4	3.5	3.0	3.0
5	4.0	4.0	4.0

[a] Average vein islet no. for apex 3.63.

TABLE II.—VIBURNUM PRUNIFOLIUM LEAF BASE[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	3.0	3.5	3.5
2	3.5	4.0	3.0
3	4.0	4.0	4.0
4	3.5	3.5	3.5
5	4.5	3.0	3.0

[a] Average vein islet no. for base 3.56.

TABLE III.—VIBURNUM PRUNIFOLIUM LEAF MARGIN-CENTER[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	3.0	3.0	3.0
2	3.0	3.0	3.0
3	3.0	3.0	2.5
4	3.0	3.0	3.0
5	4.0	2.5	4.0

[a] Average vein islet no. for margin-center is 3.06. Average vein islet number for leaf of *Viburnum prunifolium* is 3.41.

TABLE IV.—VIBURNUM RUFIDULUM LEAF APEX[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	4.0	4.0	3.5
2	5.0	4.5	3.5
3	3.5	4.5	4.5
4	3.5	4.0	4.5
5	4.5	4.0	4.5

[a] Average vein islet no. for apex is 4.13.

TABLE V.—VIBURNUM RUFIDULUM LEAF BASE[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	3.5	4.0	3.0
2	3.5	4.5	3.0
3	4.0	4.0	3.0
4	3.0	4.5	3.5
5	4.0	3.5	4.0

[a] Average vein islet no. for base is 4.1.

TABLE VI.—VIBURNUM RUFIDULUM LEAF MARGIN-CENTER[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	4.0	4.0	4.0
2	4.0	4.0	5.0
3	4.0	4.5	4.0
4	4.0	4.5	5.0
5	4.5	4.0	3.5

[a] Average vein islet no. for margin-center is 4.2. The average vein islet number for leaf of *Viburnum rufidulum* is 4.13.

TABLE VII.—VIBURNUM CASSINOIDES LEAF APEX[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	3.5	3.0	3.5
2	3.5	3.5	3.0
3	3.5	3.0	3.5
4	4.0	3.5	3.5
5	3.5	2.5	3.5

[a] Average vein islet no. for apex is 3.36.

TABLE VIII.—VIBURNUM CASSINOIDES LEAF BASE[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	3.5	5.5	3.5
2	3.5	5.5	3.0
3	3.0	3.5	3.0
4	5.0	4.5	3.5
5	4.0	4.5	3.5

[a] Average vein islet number for base is 4.

TABLE IX.—VIBURNUM CASSINOIDES LEAF MARGIN-CENTER[a]

Field	Leaf 1	Leaf 2	Leaf 3
1	4.0	3.5	3.5
2	4.0	3.5	3.5
3	4.5	4.0	5.0
4	4.0	4.0	3.0
5	4.0	3.5	3.5

[a] Average vein islet number for margin center is 3.96. The average vein islet number for the leaf of *Viburnum cassinoides* is 3.77.

STOMATAL INDEX

The stomatal index of the leaves of *Viburnum prunifolium*, *V. rufidulum*, and *V. cassinoides* was determined by removing strips of the lower epidermis of these leaves, mounting in 10 per cent of glycerin solution, counting the number of stomata and epidermal cells separately in 1 mm. square areas and applying the formula of Salisbury (5). The Whipple ocular micrometer, 4 mm. objective, and 10× ocular were employed in the counting technique. The stomatal index of one leaf of *Viburnum prunifolium* was found to be 12.6; of one leaf of *Viburnum rufidulum*, 12.27; of one leaf of *Viburnum cassinoides*, 13.3. Further determinations on additional leaves of these species are in progress.

PALISADE RATIO OF VIBURNUM LEAVES

A preliminary study was made of the palisade ratios of the leaves of *Viburnum prunifolium*, *V. rufidulum*, and *V. cassinoides*. The method followed was to cut pieces 2–3 mm. square by means of a sharp blade from the apex, margin-center, and base. The segments were placed in clearing solution consisting of 50 Gm. chloral hydrate dissolved in 20 cc. of water and gently heated for 15 minutes. The leaf pieces were then mounted in Berlese's Mountant Modified and examined under the compound microscope, using high power magnification. In each determination, the number of palisade cells under any 4 adjacent epidermal cells was divided by four, the quotient representing the palisade ratio. The results of this study for one leaf of each of the three species are as follows:

PALISADE RATIOS

Vibrunum prunifolium.—Apex, 3.2. Margin-center, 3. Base, 3. Average, 3.06.

Viburnum rufidulum.—Apex, 3.2. Margin-center, 2.7. Base 3. Average, 2.96.

Viburnum cassinoides.—Apex 3. Margin-center 3. Base, 2.5. Average, 2.83.

Further studies on the palisade ratios of these species are anticipated.

SUMMARY

1. The leaves of *Viburnum prunifolium*, *Viburnum rufidulum*, and *Viburnum cassinoides* are described as to their physical characteristics and illustrated by photographs.

2. The histology of each of these leaves is described in detail with accompanying drawings illustrating the histology of the lamina and petiole.

3. The average vein islet number values have been determined for each of these leaves. They are as follows: for *Viburnum prunifolium*, 3.41; for *Viburnum rufidulum*, 4.13; for *Viburnum cassinoides*, 3.77.

4. A preliminary report is given of the stomatal indexes and palisade ratios determined by the author for leaves of the aforementioned species. Additional work on these is in progress.

REFERENCES

(1) Youngken, H. W., THIS JOURNAL, **19**, 680(1930).
(2) Munch, J. C., Report to Scientific Section, A. PH. A., 1946, and by letter to author.
(3) Sargent, C. S., "The Silva of North America," Houghton, Mifflin and Co., Riverside Press, Cambridge, 1893, Vol. 5, p. 99.
(4) Zufall, C. J., and Burlage, H. M., THIS JOURNAL, **21**, 330(1932).
(5) Salisbury, E., *Phil. Trans. Roy. Soc.* **216B**, 1(1927).

Critical Aspects of an Acidic Germicidal Lotion*

By G. L. CHRISTENSON and ROBERT S. SHELTON†

Experiments on desirable modifications of an acidic germicidal lotion are described. The basic emulsification system of water phase, pectin, cetyl trimethyl ammonium bromide, and oleaginous phase is so modified as to improve shelf storage stability, enhance germicidal activity, decrease irritation, and improve emolliency. Critical physical, bacteriological, and pharmacological effects of ingredients such as pectin type and level, mineral oil and lanolin type and level, and bodying agents are discussed.

THE OBJECT of these studies was to determine the effect of various factors on the physical and bacteriological properties of an acidic germicidal lotion. As a result of previous work in our laboratories (1, 2) on the bactericidal efficacy of quaternary ammonium compounds and the use of such compounds as adjuncts to pectin as co-emulsifying agents, a basic formula was chosen essentially as outlined in Table I.

TABLE I.—BASIC FORMULA FOR GERMICIDAL LOTION

Mineral oil	12.00%
Lanolin (Anhydrous)	1.00
Pectin	1.00
Boric Acid	2.00
Cetyl Trimethyl Ammonium Bromide	0.16
Perfume	0.10
Distilled Water, *q. s.*, to make	100

Our experiences with this basic formula had indicated that the use of pectin with the quaternary ammonium compound, cetyl trimethyl ammonium bromide, resulted in a considerable increase in emulsification efficacy over pectin alone; thus substantiating the claims of Manchey and Schneller (3), but certain properties of the proposed emulsion necessitated this further study. The qualities which we were desirous of improving were: long-term shelf stability, germicidal activity, emolliency, corneal irritation, and odor stability.

EXPERIMENTAL

Emulsion Preparation.—Emulsions were made by preparing the water and oleaginous phases separately and combining with high-speed stirring. Emulsification was concluded by homogenization, consisting of passage through one of three machines: laboratory hand homogenizer, Manton Gaulin homogenizer, or Kom-bi-nator. Comparative studies were made on the Continental and English methods of combination and inasmuch as no significant difference in the final product was discernible, the Continental method was chosen because of its adaptability to plant operations. In this method the oleaginous ingredients were melted together at the lowest possible temperature, and the pectin was dispersed therein. This pectin-oil dispersion was then added to the water phase. The resulting emulsion was agitated continuously until the pectin was completely hydrated, after which the preparation was homogenized.

Centrifuge Stability.—Samples were then subjected to an accelerated centrifuge test consisting of rotation of sample at 3600 r. p. m. in a 10-inch angle head centrifuge for one hour. Attempts were made to measure the per cent of creaming as induced by this treatment, but because of the varying demarcation of cream layer, it was impossible to measure accurately the more stable preparations. This procedure does eliminate the more unstable samples and its use in this regard was valuable.

Storage Conditions.—Samples which survived the centrifugation test were stored at the various temperatures routinely employed in our product development studies (4°, room temperature, 37°, and 45°). These stored samples were examined at weekly intervals for the first month and thereafter at six-month and one-year intervals. From the sales and distribution standpoint, we feel that a product of the liquid emulsion type should show no change in physical properties for at least one year at room temperature and 4°. Slight creaming is allowable at 37° at the six-month period if no change in room temperature samples occurs in one year. Samples must be stable at 45° for at least one month and meet the foregoing requirements to be considered stable. These are rigid standards, but past experience has shown that they are minimal for a product of this nature to maintain its elegance under all distributional conditions.

Viscosity Determination.—Viscosity of preparations was determined by a pipette method, and is recorded as viscosity relative to water. For these studies the tip of a 10-cc. pipette was cut off and fire-polished so that the water delivery time was 2.8 seconds. The time required for emptying of a given sample, divided by the water delivery time, is the relative viscosity. No attempt was made to apply the minor corrections involved, but since these determinations were all made at 30°, and since the viscosity of water at 25° is 0.00805 poises, for all practical and comparative purposes one relative viscosity unit is equal to one centipoise unit.

Oil Globule Determination.—Oil globule counts were made by mixing an accurately weighed sample with known volume of melted 5% gelatin solution and spreading a measured amount of this dispersion over an area of exactly 1 sq. cm. on a microscope slide. This slide is allowed to harden in the

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refrigerator to retard Brownian movement and is then examined at 600 × magnification with a calibrated eyepiece. By averaging the number of globules per given area in various portions of the field an accurate transposition of number of globules per mg. of original emulsion can readily be made.

Germicidal Evaluation.—Critical Killing Time determinations were conducted in inoculating the lotion, "as is," with vegetative growth of the indicated test organism. Unless otherwise indicated, *Staphylococcus aureus* was employed as the test organism. Subcultures were then made from the inoculated lotion into "Letheen" broth after the indicated time intervals to inactivate any residual quaternary ammonium compound (4). Results of duplicate tests are recorded. Himebaugh cup determinations, consisting of measurement of zone of inhibition of growth of test organism in agar media, were also made. In view of the work of Quisno and Foter (5) on the decrease of germicidal activity of quaternary ammonium compounds by agar, we do not consider these data indicative of the true activity of quaternary ammonium compounds, and no attempt is made to correlate them with the other data reported.

RESULTS

As indicated previously, no difference was found in formulation of the basic formula by Continental or English methods. The method of homogenization did cause significant differences in emulsion quality. The differences in homogenization efficiency of three types of homogenization tools are shown in Table II. These data show that the hand laboratory homogenizer is better than no homogenization, but is not comparable to the two commercial machines employed. The Kom-bi-Nator is remarkably efficient as a homogenizing machine for this type of preparation.

A number of different types of pectin were employed in the basic formula as shown in Table III. From these data it is evident that there is considerable difference in pectins as co-emulsifiers for this basic emulsion. In two cases it was not possible to obtain satisfactory emulsions initially. Low-methoxy pectin resulted in satisfactory emulsification, but because of the thinness of preparation, some creaming occurred in six months. Stability of preparations containing the 210C No. 8 and 100 gel strength pectins was similar to that of the low-methoxy pectin preparations. All three lots of Pectin N. F. employed produced a satisfactorily stable emulsion. The data shown in this table also are illustrative of the previously mentioned lack of complete correlation between the accelerated centrifuge stability test and long-term shelf stability results.

TABLE II.—EFFECT OF TYPE OF HOMOGENIZER ON EMULSION QUALITY

Type Homogenizer	Pressure Employed	Oil Globule Count, Millions per Mg.
None	.....	6
Hand Homogenizer[a]	?	55
Manton Gaulin[b]	2000 lbs. per sq. in.	380
Kom-Bi-Nator[c]	750 lbs. per sq. in.	1323

[a] Hand model—American Profession Pharmacist.
[b] Type 25CGB—Manton Gaulin Mfg. Co.
[c] Model K-200—Marco Co., Inc.

We were further interested in studying the effect of variation of certain ingredients in the basic formula on viscosity and Critical Killing Time of the lotion. In Table IV the effect of variation of mineral oil-lanolin levels and pectin levels on relative viscosity and Critical Killing Time is shown. The changes due to variation of pectin level are in the order expected—an increase in viscosity and no difference in Critical Killing Time. However, in the case of increasing levels of oleaginous phase, rather surprising increases in viscosity were encountered along with unexpected prolongation of Critical Killing Time. We have hypothesized that this phenomenon is a function of alteration of the degree of emulsification. The abnormal rise in viscosity is attributed to an increased rigidity at the globule interfaces and the decrease in Critical Killing Time to inaccessibility of the quaternary ammonium compound because of its tie-up at the globule interfaces. The effect is presented in somewhat clearer fashion in Fig. 1.

TABLE III.—EFFECT OF VARIOUS PECTINS ON EMULSION PROPERTIES

Pectin Employed	Relative Viscosity	Critical Killing Time, Min.	Centrifuge Stability	Shelf Stability
Low Methoxy—Citrus	2.0	3–5	OK	Slight creaming at six months
Pectin NF—Lot A	9.1	3–5	OK	OK at one year
Pectin NF—Lot B	9.0	3–5	OK	OK at one year
Pectin NF—Lot C	8.2	5	OK	OK at one year
100 Gel Strength—Apple	1.5	3–5	Creamed excessively	Creamed and separated at one month
150 Gel Strength—Apple	Emulsion very dark and thin—separated readily—no further study made			.....
Rx Grade—No. 300—Apple	Emulsion curdled—unsatisfactory			.....
Rx Grade—No. 3—Na Salts—Apple	5.5	10	OK	Creamed and separated at six months
210C No. 8—Apple	5.8	10	OK	Slight creaming at one month, creamed at six months

Since we were interested in retaining germicidal activity while increasing stability, viscosity, and emolliency, the data here appeared to preclude achievement of all goals. Various adjuncts to the oleaginous phase such as glyceryl mono-stearate glyceryl mono-oleate, hydrogenated vegetable oils, and polyoxyalkalene derivatives were tested without achieving all of the desired goals. Attempts to modify the water phase by polyethylene glycols resulted in undesirable emulsion characteristics but the use of methyl cellulose as a bodying agent gave promising results. It was found that although methyl cellulose alone would not satisfactorily replace pectin in the formulation, its use as an adjunct to pectin resulted in increased viscosity without detracting from the desired bacteriological qualities. With the aid of this agent we were able to make preparations of any desired viscosity.

At the time this work was done, the quality of lanolin commercially available was extremely variable. Studies on various grades of lanolin revealed that the more highly purified grades were the most satisfactory. Apparently, impurities in regular anhydrous Lanolin U. S. P. were instrumental to some degree in inactivation of the germicidal action of cetyl trimethyl ammonium bromide. Cosmetic grade lanolin was selected as free from this inactivation phenomenon and the effect of varying levels of this material in the formulation was tested. As shown in Table V, levels of 2.4% to 3.6% of this material were optimal in so far as emulsion stability and Critical Killing Time were concerned. Increase in concentration of this material also resulted in improvement of emolliency, a quality which is difficult to express objectively. Preparations were tested comparatively by application of equal amounts to a 4-sq. in. skin area, and observing effects at application and after fifteen- and thirty-minute intervals. The bland aroma of this material also resolved, to a considerable degree, the question of perfume perception and stability which was initially a problem.

The basic formula was initially submitted to our Pharmacology Department for rabbit corneal irritation tests. This test involves direct application to

TABLE IV.—EFFECT OF VARIATION OF PECTIN AND OIL ON LOTION CHARACTERISTICS

Sample	Mineral Oil/Lanolin Ratio	Pectin/Cetyl Trimethyl Ammonium Bromide Ratio	Water/Pectin Ratio	Oil/Pectin Ratio	Pectin, %	Mineral Oil Lanolin, %	Relative Viscosity	Critical Killing Time, Min.
A	11.7	6.4	87.7	9.0	1.0	9.0	4.4	10–20
B	11.7	6.4	81.8	14.0	1.0	14.0	5.9	20
C	11.7	6.4	78.8	18.0	1.0	18.0	7.6	20
D	11.7	6.4	72.8	24.0	1.0	24.0	22.4	20–30
E	11.7	6.4	60.8	36.0	1.0	36.0	52.4	60
F	11.7	5.0	106.0	15.7	0.8	12.6	2.7	10–20
G	11.7	6.0	94.8	14.0	0.9	12.6	4.6	10–20
H	11.7	6.2	85.2	12.6	1.0	12.6	5.6	10–20
I	11.7	7.5	71.0	10.5	1.2	12.6	7.9	10–20
J	11.7	8.9	60.7	9.0	1.4	12.6	9.5	10–20

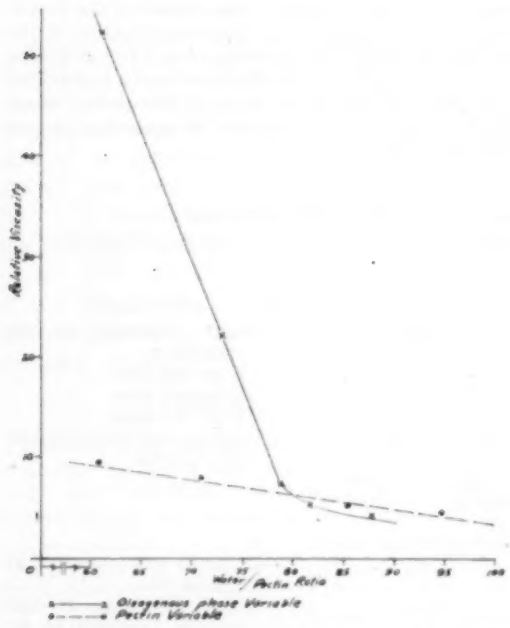

Fig. 1.—Effect of Variation of Oil and Pectin on Relative Viscosity.

TABLE V.—EFFECT OF VARIATION IN LANOLIN LEVEL ON LOTION PROPERTIES

% Lanolin (Cosmetic Grade)	Critical Killing Time, Min.	Stability
None	<3	Curdled in three weeks at room temperature
0.3	3	Curdled in one month at room temperature
0.6	<3	Slight creaming, curdled in six months at 37°. OK for one year at room temperature
1.2	3	Slight creaming, curdled in six months at 37°. OK for one year at room temperature
2.4	<3	OK for one year at all temperatures
3.6	3	OK for one year at all temperatures
4.8	<3	Slight creaming in one year at room temperature

TABLE VI.—GERMICIDAL EFFICIENCY OF BABY LOTION

Organism	No Serum						10% Added Serum					
	3′	5′	10′	20′	30′	60′	3′	5′	10′	20′	30′	60′
Staphylococcus aureus	+ +	− −	− −	− −	− −	− −	+ +	+ −	− −	− −	− −	− −
Eberthella typhosa	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −
Streptococcus viridans	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −
Staphylococcus albus	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −
Streptococcus hemolyticus	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −
Escherichia coli	+ +	+ +	+ −	− −	− −	− −	+ +	+ +	+ +	+ +	+ −	− −
Pseudomonas aeruginosa	+ +	+ +	− −	− −	− −	− −	+ +	+ +	+ +	+ +	− −	− −

+ Indicates growth of the organism after subculturing.
− Indicates no growth of the organism after subculturing.

the cornea and is an acute and severe test; but since our aim was to produce a satisfactory baby lotion, we felt that little irritation should be manifest in this severe test in view of the proposed application to the tender skin of the newborn infant. The basic lotion was severely irritating as measured by rabbit corneal application. After embodiment of several changes in the formulation (methyl cellulose incorporation, mineral oil increase, lanolin increase and change in quality, and modification of perfume) samples were resubmitted and found to exert very slight, if any, transient irritation.

Also, after incorporation of the changes previously described, the lotion was submitted to the Bacteriology Department for germicidal evaluation against a number of organisms commonly encountered on the skin. Their results are shown in Table VI. These results indicate that the lotion possesses remarkably good germicidal properties and, in view of its intermittent periodic application, might reasonably be expected to keep the bacterial flora of the skin surface to which it is applied at a minimum.

Clinical studies have further substantiated our belief that the emulsion system results in a therapeutically efficacious, mild, and soothing lotion. Preliminary studies (6) have indicated that it is an excellent agent for prevention and cure of impetigo epidemics among newborn infants.

SUMMARY

Experiments on desirable modifications of an acidic germicidal lotion are described. The basic emulsification system of pectin and cetyl trimethyl ammonium bromide is modified to improve shelf storage stability, enhance germicidal activity, decrease irritation, and improve emolliency. Critical aspects of the various ingredients such as pectin type and level, mineral oil and lanolin type and level, and bodying agents are discussed.

REFERENCES

(1) Shelton, Robert S., assignor to Wm. S. Merrell Company, U. S. Pats. 2,295,504 and 2,295,505.
(2) Shelton, R. S., Van Campen, M. G., Tilford, C. H., Lang, H. C., Nisonger, L., Bandelin, F. J., and Rubenkoenig, H. L., *J. Am. Chem. Soc.*, **68**, 753(1946).
(3) Manchey, Lessel Lavan, and Schneller, George H., assignors to Vick Chemical Company, U. S. Pat. 2,372,159.
(4) Quisno, R., Gibby, Irvin W., and Foter, Milton J., *Am. J. Pharm.*, **118**, 320(1946).
(5) Quisno, Robert A., Gibby, Irvin W., and Foter, Milton J., THIS JOURNAL, **35**, 317(1946).
(6) Fischer, Carl C., *Arch. Pediat.*, **61**, 352–7 (1944).

The Turbidimetric Determination of Peptic Activity*

H. J. WESSELMAN and W. W. HILTY†

The use of crystallized egg albumen as a new substrate is described in a rapid method for the determination of peptic activity. The over-all time consumed in the assay of a series of pepsin samples represents approximately one-third of the time that is required for the officially described N. F. procedure. The accuracy is improved by this method and the variations in the substrate that have been so frequently observed are practically eliminated.

BECAUSE of the variation in the egg albumen used in the standardization of pepsin preparations as directed in the National Formulary VIII (1), it has not always been possible to obtain good digestion of the substrate and thus it has been very difficult to determine the relative strength of pepsin preparations from time to time. There are many factors which could account for this variation, none of which can be controlled satisfactorily by the analyst interested in determining the potency of the preparations in question.

A number of approaches to the standardization of pepsin were considered. However, due to the complexity of the compound and the nature of its action, the most logical procedure still remains that of digesting some form of protein. Based on this assumption our efforts were then directed toward the development of a substrate

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which would eliminate most of the variable factors.

The literature contains many references (2–6) on determining pepsin in body fluids and in these a number of methods of approach are described. There are also various methods reported for crystallizing egg albumen. This appeared to open the way for the preparation of a more stable and more uniform substrate than has previously been used.

EXPERIMENTAL

A crystallized egg albumen was prepared in accordance with the method suggested by Cole (7). The solution thus obtained was further treated by freezing and then drying in a vacuum. This preparation is very soluble in water and a 1% solution when heated in a boiling water bath for fifteen minutes to coagulate the protein yields a very uniform and finely divided suspension. This suspension can be reproduced and is stable for periods of at least one month when stored in a refrigerator.

The availability of the uniform and finely divided suspension suggested the possibility of determining the unused portion of the substrate by a photoelectric measurement of the turbidity of the digested sample. To accomplish this it was necessary to determine whether or not the rate of digestion would proceed in a uniform manner and if the reaction could be terminated for a sufficient length of time to allow an accurate reading to be taken. Results as shown in Table I and illustrated graphically in Fig. 1 indicate that the reaction does proceed uniformly for approximately fifty minutes and from those shown in Table II and in Fig. 2 it is observed that the immediate chilling of the reaction mixture will completely retard the peptic action for at least one-half hour. This is ample time to permit the necessary readings on an instrument equipped to make turbidimetric readings.

TABLE I.—RATE OF DIGESTION DETERMINED BY INCREASE IN GALVANOMETER READING PER UNIT TIME

Time, Min.	A 3.33 Mg. N. F. Reference Pepsin	B 3.33 Mg. N. F. Reference Pepsin	C 3.33 Mg. Pepsin 1:3000	D 3.33 Mg. Pepsin 1:3000
0	34.5	34.5	34.5	34.5
5	38.0	38.0	38.0	38.0
10	40.0	40.0	40.0	40.0
15	42.5	41.5	42.5	42.5
20	44.0	43.0	45.0	44.5
25	46.5	45.0	47.5	47.5
30	50.0	47.5	52.5	51.5
35	52.0	51.5	56.5	55.0
40	56.5	56.0	61.0	60.0
45	60.0	59.5	68.0	66.0
50	67.0	64.0	78.5	76.0
55	73.0	70.0	89.5	85.5
60	81.0	77.0	+100.0	99.0

Reagents

(*1*) *0.3% HCl.*—Mix 35 cc. of 1 *N* hydrochloric acid with 385 cc. of distilled water.

(*2*) *Albumen Substrate.*—Prepare a crystallized egg albumen as suggested by Cole (7). Further treat the solution thus obtained by freezing and then drying in a vacuum. Dissolve 1.0 Gm. of crystalline egg albumen in 100 cc. of distilled water. Heat in boiling water bath for fifteen minutes with constant agitation. Strain through muslin into 100-cc. volumetric flask, cool, and add sufficient distilled water to make 100 cc.

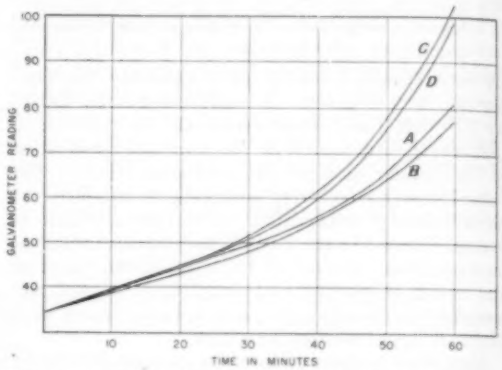

Fig. 1.—Rate of digestion determined by increase in galvanometer reading per unit time.

TABLE II.—EFFECT OF CHILLING REACTION MIXTURE

Time, Min.	A 3.33 Mg. N. F. Reference Pepsin	B 3.33 Mg. Pepsin 1:3000	C 2.86 Mg. Pepsin 1:3000
52° C.			
0	34.5	34.5	34.5
45	60	55	48
Ice Bath			
5	60	57	49
15	60	56	49
30	60.5	56	49.5
45	60	56	49.0
55	60	56	49.0

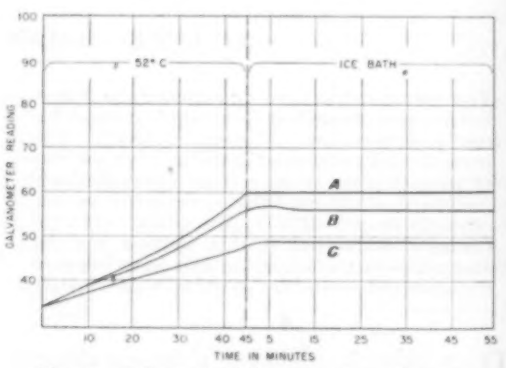

Fig. 2.—Effect of chilling reaction mixture.

(*3*) *N. F. Reference Pepsin.*—(A) Dissolve 0.1000 Gm. of N. F. Reference Pepsin in sufficient 0.3% HCl to make 100 cc.

(B) Transfer 10 cc. of solution A to a 100-cc. volumetric flask and dilute to 100 cc. with 0.3%

TABLE III.—COMPARISON OF RESULTS

Sample	N. F. VIII Method Standard Cc. Residue	N. F. VIII Method Sample Cc. Residue	N. F. VIII Method Relative Strength	Proposed Method Standard Reading	Proposed Method Sample Reading	Proposed Method Relative Strength
A						
1:3000	18.5	15.0	+1:3000	60	63.0	+1:3000
1:3500	18.5	18.0	+1:3500	60	54.0	−1:3500
B						
1:3000	18.5	14.0	+1:3000	60	61.0	+1:3000
1:3500	18.5	19.0	−1:3500	60	55.0	−1:3500
C						
1:3000	18.5	12.0	+1:3000	60	63.0	+1:3000
1:3500	18.5	18.0	+1:3500	60	56.5	−1:3500
D						
1:3000	18.5	15.0	+1:3000	60	63.5	+1:3000
1:3500	18.5	21.0	−1:3500	60	58.0	−1:3500
E						
1:3000	27.0	26.0	+1:3000	60	61.0	+1:3000
1:3500	27.0	25.0	+1:3500	60	60.0	1:3500
F						
1:3000	27.0	24.0	+1:3000	60	71.0	+1:3000
1:3500	27.0	26.0	+1:3500	60	66.0	+1:3500

HCl. Each cc. is equivalent to 0.10 mg. of N. F. Reference Pepsin.

Apparatus

(*1*) *Constant Temperature Bath.*—Water bath thermostatically controlled to maintain a temperature of 52° ± 1°.

(*2*) *Turbidimeter.*—A photoelectric instrument capable of making turbidimetric readings. In this work we have used a Pfaltz and Bauer Fluorophotometer Model B using a light source of 440 mμ.

(*3*) *20-Cc. Matched Pyrex Test Tubes.*—Clean test tubes with acid cleaning mixture and dry thoroughly.

Procedure

Transfer exactly 5 cc. of the albumen substrate to each of three test tubes marked *B*, B_1, and B_2. To one of three matched 20-cc. Pyrex test tubes marked *A* add exactly 5 cc. of 0.3% HCl solution of Pepsin containing 0.10 mg. of Pepsin 1:3000 per cc. To the second matched Pyrex test tube marked A_1 add exactly 4.30 cc. of a 0.3% HCl solution of Pepsin containing 0.10 mg. of Pepsin 1:3000 per cc. and 0.70 cc. of 0.3% HCl. To the third matched Pyrex test tube marked A_2 add exactly 5 cc. of Reagent N. F. Reference Pepsin (Solution B). Pour the contents of tube *B* into tube *A*, the mixture into tube *B*, and back to tube *A*. Repeat this mixing process with each pair of tubes.

When mixing is complete immerse the tubes in a water bath so that the level of the contents of the tubes is below that of the water. Maintain a temperature of 52° for forty-five minutes. At the end of this period quickly remove the tubes and place them in an ice water bath for at least five minutes.

Place the tube containing the N. F. Reference Standard in a Pfaltz and Bauer Fluorophotometer and set the galvanometer reading at 60 using a light source of 440 mμ. The tube containing 5 cc. of the solution of Pepsin being assayed should read not less than 60 and the tube containing 4.30 cc. of the solution of pepsin being assayed should not read more than 60 on the galvanometer.

Table III shows a comparison of the present method and the proposed method.

In the event the sample being assayed is stronger or weaker than the Reference Standard a true evaluation can be obtained by using a smaller or larger aliquot of the sample and repeating the assay.

CONCLUSION

A rapid method for standardizing pepsin involving a modified substrate and procedure is presented in which accuracy is improved and closer evaluation is possible.

REFERENCES

(1) National Formulary, Eighth Edition, Mack Printing Co., Easton, Pa., 1946, p. 379.
(2) Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, **16**, 59(1932).
(3) Riggs, B. C., and Stadie, W. C., *J. Biol. Chem.*, **150**, 463(1943).
(4) Northrop, J. H., *J. Gen. Physiol.*, **16**, 41(1932).
(5) Hecht, M., and Civin, H., *J. Biol. Chem.*, **116**, 477 (1936).
(6) Albanese, A. A., Irby, V., and Saur, B., *ibid.*, **166**, 231(1946).
(7) Cole, A. G., *Proc. Soc. Exptl. Biol. Med.*, **30**, 1162 (1933).

Studies on the Camphor Basil, *Ocimum Kilimandscharicum* Gürke*

By HEBER W. YOUNGKEN and WILLIAM E. HASSAN, Jr.

Detailed descriptions of the histology of the stem, leaf, bracts, and flowering parts of the Camphor Basil are presented. The yield of camphor is also recorded.

THE PUBLISHED report by Lowman and Kelly in 1945 (2) disclosing the results of their investigations of the Camphor Basil which they grew successfully on an experimental scale at Arlington Farms, Va., and at Beltsville, Md., and from which they isolated a camphor in purified form having physical and chemical properties similar to natural camphor (from *Cinnamomum Camphora*) incited the interest of the authors whose objectives were to determine the habit, the anatomical characteristics, and chemical properties of *Ocimum kilimandscharicum* Gürke as cultivated in New England (Fig. 1).

Fig. 1.—Camphor Basil, *Ocimum kilimandscharicum* Gürke, × 1/3.

Seeds of the Camphor Basil were generously supplied by Dr. Lowman of the U. S. Department of Agriculture Horticultural Station at Beltsville, Md. These seeds were planted in the greenhouse of the Arnold Arboretum at Jamaica Plain, Boston, Mass. The seedlings were then transplanted, in early May, to the Medicinal Plant Garden of the Massachusetts College of Pharmacy, where they were grown in moderately limed soil having a *p*H of 5.57. Crops were planted and harvested in 1946 and 1947. Not a plant was lost in the garden, all persisting in a healthy condition through the flowering and fruiting stages. Observations were made on these plants during the flowering and fruiting periods, studied in the College laboratories, and some new facts ascertained regarding their morphology. The general taxonomic characteristics of these plants agreed with the original description of the species found in the Kenya Colony by Gürke (3), except that they were annual in habit.

The purpose of this paper is to report the results of our investigation on the growth habit and structure of *Ocimum kilimandscharicum* cultivated in Boston, as well as the results of preliminary studies made on the oil and camphor distilled from the aerial portion of this cultivated species gathered on October 25, 1947.

GROWTH HABIT AND GROSS MORPHOLOGY

While the Camphor Basil, as described by Gürke (3), is a shrubby plant in its East African habitat, it is an annual in the latitude of Boston where the plants possessed the general characteristics of grayish green, bushy herbs attaining a height of from 1 1/2 to 2 1/2 ft. From a woody fibrous root system arises a brownish, hairy, subquadrangular stem with many branches beginning near the base. The branches are villose with white hairs and possess numerous petiolate, pubescent to villose leaves and terminal, pubescent, spicate racemes of verticillasters.

The leaves are ovate to ovate-lanceolate to broadly elliptic and measure up to 6.6 cm. in length, the petioles up to 1.5 cm. long, and the lamina from 1.0 to 2.7 cm. wide. These leaves are dull green in color and exhibit an acute summit, a cuneate base, a crenate-serrate to serrate margin in the upper portion of the leaves, and an upper and lower surface

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Presented to the Pharmacy Subsection of the Medical Sciences Section of the American Association for the Advancement of Science, Chicago meeting, 1948.

which are appressed, hairy, and glandular-punctate. Upon crushing or bruising the leaves, a strong camphor odor is emitted.

The flowers occur in 4- to 6-flowered whorls, each whorl subtended by 2 small bracts on long villose racemes, the latter up to 28 cm. in length. Each flower consists of a greenish purple, campanulate, bi-labiate calyx up to 4 mm. in length whose upper lip is orbicular with a mucronate apex and the lower lip 4-toothed, the two lower teeth subulate, longer and narrower than the lateral, broadly ovate to ovate-acuminate teeth.

The corolla is tubular-bilabiate and measures from 7 to 10 mm. in length, the upper lip weakly rose-red with white, ciliate, hairy margins, the lower lip white and ciliate-hairy on the lower face.

The 4 didynamous stamens possess long filaments, the upper filaments with a downward curved, hairy appendage near the base. The anthers are versatile. The style is long, exserted, and terminates in 2 digit-like style arms.

The nutlet fruits are ovoid to ovoid-oblong, rounded at one end, truncate to narrowly rounded at the other, and exhibit an elongated, triangular, flattened area on one side. These nutlets possess a black to brown testa and measure up to 1.6 mm. in length.

Two bracts subtend each verticillaster. They are short-petiolate, ovate, with cuspidate apex, entire margin and rounded tapering base. Their surfaces and margins are villose and glandular hairy. Those measured on one raceme were up to 10 mm. long and 5 mm. wide.

HISTOLOGY OF THE STEM

Transverse sections of the stem (Fig. 2) of *Ocimum kilimandscharicum* are subquadrangular in outline and present the following characteristics:

(*a*) Epidermis of more or less rounded cells covered by a prominent cuticle and bearing nonglandular and glandular hairs. The nonglandular hairs are of two types, *viz.*, short, unicellular, conical hairs which arise from 1, 2, or 3 epidermal cells and uniseriate, papillose hairs with a usually short, broad, basal cell and pointed end cell. The latter type is usually composed of 5 to 6 cells but occasionally 7- to 8-celled hairs have been found. The glandular hairs possess a one-celled stalk and a head of 1, 2, 4, or 8 secretory cells.

(*b*) Cortex of about 5 layers of cells, except in the rib zones where it is broader. Just beneath the epidermis, collenchyma occurs which constitutes a broad zone in the rib regions and a narrow zone in the intercostal regions. Beneath this occur tangentially elongated, ovoid, cortical parenchyma cells.

(*c*) Endodermis, contrary to Blanque and Maheu (1) who are stated to have wrongly identified this species as *Ocimum canum*, we have found endodermis differentiated in the younger portion of the stem into a prominent layer of clear, more or less radially elongated cells. In older portions of the stem, however, this differentiation is lost and the endodermal cells appear tangentially elongated.

(*d*) Pericycle, a zone of parenchyma in which are imbedded oval to irregularly oblong groups of pericyclic fibers forming an interrupted circle. These groups are largest in the rib regions. The fibers possess a thin wall and a broad lumen. They are nonlignified in young parts of the stem, becoming lignified in the older, thicker portions.

(*e*) Open collateral fibrovascular bundles consisting of a narrow phloem of sieve tissue devoid of fibers. A prominent cambium is present and a relatively broad xylem of narrow wood wedges and wood rays. The wood wedges are composed of lignified wood fibers and tracheae, the latter arranged in radiate fashion and most numerous in the early wood.

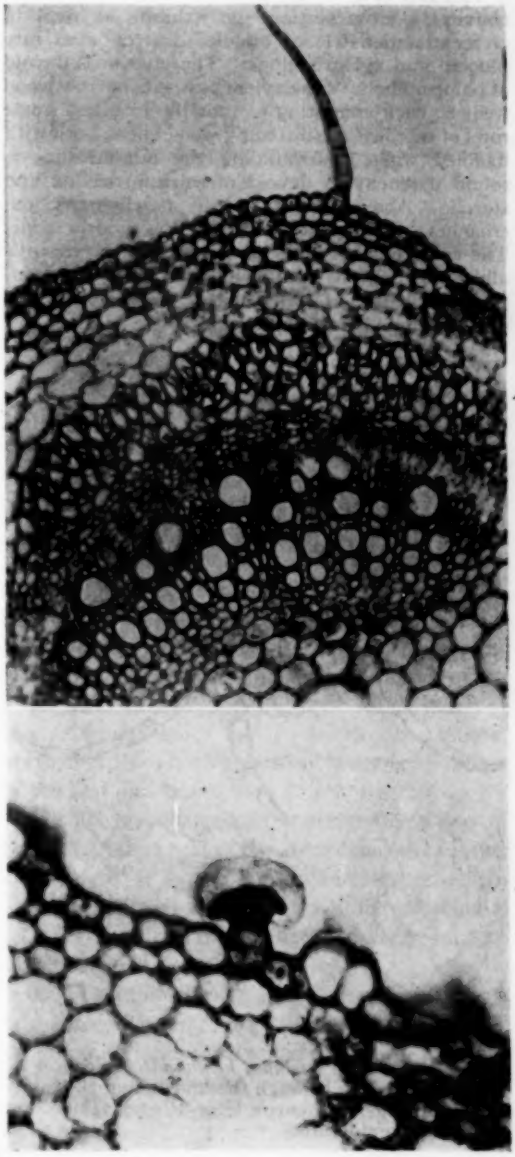

Fig. 2.—*A*, transverse section of the stem of *Ocimum kilimandscharicum*, × 100. *B*, transverse section of outer portion of the same stem showing a glandular hair, × 200.

(*f*) Pith, a broad central zone of pitted parenchyma.

In longitudinal sections, the tracheae and the tracheids exhibit spiral and pitted markings.

HISTOLOGY OF THE LEAF BLADE

This leaf (Fig. 3) has a midrib which is concavo-convex in cross section and exhibits an oval to crescentic meristele of bundles differentiated into phloem and xylem regions. The phloem is devoid of phloem fibers. The xylem possesses narrow wood wedges, each consisting of radially arranged single rows of tracheae separated by wood fibers, both with lignified walls. Surrounding the bundle zone is round parenchyma devoid of calcium oxalate and starch. Above, the palisade parenchyma encroaches slightly laterally into the midrib zone. The epidermis is composed of rounded to tangentially elongated cells from which arise uniseriate, nonglandular hairs. The midrib is reinforced by a layer of collenchyma beneath the epidermis.

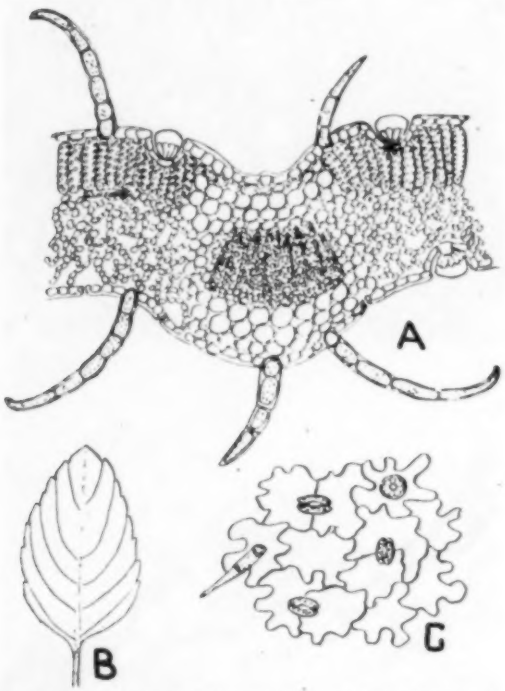

Fig. 3.—Leaf of *Ocimum kilimandscharicum*. *A*, transverse section through the midrib and the interneutral regions. *B*, entire leaf. *C*, surface section of the lower epidermis.

The interneural region of the lamina exhibits a layer of epidermal cells on both surfaces which are somewhat rounded to slightly tangentially elongated in cross section and deeply sinuate wavy in surface view. The cells of the lower epidermis are smaller than those of the upper epidermis. Ovate to elliptical stomata occur, in both epidermises, which measure up to 42 μ in length. They are of the Labiate type. A cuticle covers the epidermal cells. Both epidermises show numerous crypts from the base of each of which arise sessile or short-stalked hairs, the glandular heads of which exhibit from 4 to 8, occasionally 12, secretory cells and an upraised subglobular, to balloon-shaped cuticle. Between the cells of the gland and the cuticle, oil and camphor are deposited. The cells lining the crypt are tangentially elongated in cross section. In addition, both epidermises possess numerous nonglandular hairs mostly of the uniseriate, papillose type. While most of the hairs found are composed of 5 to 6 cells, a few have been found up to 7 and 8 cells. The basal cell of each hair is usually broad and the distal cell, narrow and pointed. Some of the nonglandular hairs measured up to 1350 μ in length.

Beneath the upper epidermis there occurs a single layer of palisade parenchyma which occupies in different regions from one-third to one-half the mesophyll. The spongy parenchyma is composed of irregularly rounded to elliptic cells forming an open network of cells surrounding prominent air spaces. Just above the stomata of the lower epidermis, the spongy parenchyma cells surround large substomal air spaces. The vascular strands coursing through the spongy parenchyma exhibit tracheary elements with spiral thickenings.

TABLE I.—MOISTURE CONTENT[a]

Sample No.	Wt. of Sample, Gm.	Wt. of Sample After Drying	% Moisture
1	98.7	26.2	73.4
2	95.4	21.4	77.5
3	97.2	23.8	75.5
4	97.9	23.7	75.7
Av.	97.3	23.8	75.5

[a] The moisture content was determined by cutting the drug up into small pieces, placing in large evaporating dishes, and placing in a 37° constant temperature oven until the sample attained constant weight.

TABLE III.—PHYSICAL CHARACTERISTICS OF THE OIL

Appearance	Yellowish liquid
Odor	Camphoraceous
Specific gravity	0.9225 at 25°
Refractive index[a]	1.4752 at 20° 1.4735 at 25°

[a] The refractive index was determined according to the method described in the U. S. P. XIII (5).

TABLE IV.—PHYSICAL CHARACTERISTICS OF THE CAMPHORACEOUS DEPOSIT AND ITS DERIVATIVES

Appearance	White, crystalline, granular mass
Odor	Resembling that of camphor
Taste	Aromatic and pungent
Solubility	Insoluble in water 1 Gm. soluble in 1 cc. alcohol 1 Gm. soluble in 0.5 cc. chloroform Readily soluble in the extracted oil
Melting point	174 to 176°
M. p. of oxime	116°
M. p. of semicarbazone[a]	234°

[a] The derivatives were prepared according to the methods of Shriner and Fuson (4) and the melting points according to the U. S. P. XIII (6).

TABLE II.—CAMPHOR AND OIL CONTENT*

Sample No.	Wt., Gm.	Time of Distn., Hr.	Part Used	Camphor, %	Oil, %
1	70.0	3.5	Entire fresh herb	0.98	0.43
2	70.0	4.0	Entire fresh herb	0.83	0.36
3	50.0	4.0	Leaves and flowering tops dried at 37° for 48 hr.	2.15	2.20
4	50.0	4.0		2.12	2.00
5	50.0	4.0		2.91	2.80
6	50.0	4.0		3.01	3.00

Av. Camphor Content, dried leaves and flowering tops, 2.54%.
Av. Oil Content, 2.50%.

* The oil was collected by the U. S. P. method (7) for collecting oils lighter than water and was freed from any camphor in solution by freezing until no more camphor crystallized out.
The camphor was collected on the condenser of the above apparatus, removed by dissolving in chloroform, recrystallized, and dried over sulfuric acid for forty-eight hours.

HISTOLOGY OF THE FLORAL PARTS AND BRACTS

The calyx, corolla, and bracts possess epidermal cells with curvilinear vertical walls, stomata, and papillose, nonglandular hairs similar to those which occur on the leaves except that there are more of the 7- and 8-celled, uniseriate type. These hairs are up to 1460 μ in length. The glandular hairs show mostly 4 secretory cells, are stalked, and not sunken in crypts in the corolla. The bract shows three nerves, a ciliate margin, and its apex is pilose-hairy.

The filaments of the stamens consist of (*a*) an epidermis of elongated epidermal cells having thin walls and containing minute yellowish, globoid to ovoid bodies of protein, occurring in aggregates within the nuclei, and irregularly shaped chromoplastids in their cytoplasm; (*b*) a narrow zone of elongated, thin-walled parenchyma; (*c*) a central vascular strand of sieve tissue and spiral tracheae. Just above the insertion point of the filaments, they are covered with numerous stout, papillose, unicellular, uniseriate, nonglandular hairs, the latter consisting of up to 3 cells, conspicuously jointed, the end cell either obtuse or pointed.

The pollen grains are subspheroidal with a reticulate exine and measure up to 72 μ in diameter.

CONSTITUENTS

Lowman and Kelly (2) in their report gave figures for the yield of oil and camphor which had been extracted from material gathered at various times during the summer. The figures stated below are for material which was extracted from plants gathered just before frost on October 25, 1947.

The physical constants of the camphor and oil and data pertaining to the moisture, camphor, and oil content are recorded in Tables I–IV.

SUMMARY

1. Camphor Basil, *Ocimum kilimandscharicum*, possesses the growth habit of an annual herb in the latitude of Boston, Mass., where the species can be grown to maturity if planted in early spring.

2. Plants of Camphor Basil grown in the Medicinal Plant Garden of the Massachusetts College of Pharmacy are described. These were found to agree in their essential taxonomic characters with the description of the species by Gürke and Engler.

3. Detailed descriptions are given of the histology of the stem, leaf, bracts, and flowering parts, and new facts reported on their microscopic structure.

4. The chief diagnostic histological elements of the stem, leaf, and bract are as follows: (*a*) uniseriate, papillose, nonglandular hairs, usually of up to 5 or 6 cells but occasionally of 7 or 8 cells, each with a short, broad, basal cell and a narrow, pointed, distal cell; (*b*) short, conical, papillose, 1- to 2-celled hairs; (*c*) sessile to short-stalked glandular hairs with heads of 1, 2, 4, or 8 secretory cells and an upraised cuticle, many of which contain camphor and volatile oil; (*d*) numerous crypts occurring on the epidermis of the foliage leaves from the bases of which some of the glandular hairs arise; (*e*) deeply sinuate, vertical walls of both upper and lower epidermises of the leaf and bract.

5. The endodermis in transverse sections of the younger portion of the stem consists of large, clear, more or less radiately elongated cells. In older portions of the stem this differentiation is lost, the endodermal cells there appearing tangentially elongated.

6. A camphor and oil are present in *Ocimum kilimandscharicum* each of which possesses physical characteristics similar to the natural camphor and oil obtained from *Cinnamomum Camphora*.

7. The dried leaves and flowering tops of the Camphor Basil grown in Boston and harvested in late October yielded an average of 2.5% of oil and 2.54% camphor.

REFERENCES

(1) Blanque, G., and Maheu, J., *Compt. rend. acad. agr. France*, 13, 1102–10(1927).
(2) Lowman, M. S., and Kelly, J. W., THIS JOURNAL, 35, 153–62(1945).
(3) Gürke in Engler, A., "Die Pflanzenwelt Ost Africa," Theil, A., C. D. Riemer, Berlin, 1895, pp. 349–50.
(4) Shriner, R. L., and Fuson, R. C., "Indentification of Organic Compounds," John Wiley & Sons, New York, 1935, p. 145.
(5) United States Pharmacopœia XIII, Mack Printing Co., Easton, Pa., 1947, p. 682.
(6) *Ibid.*, p. 667.
(7) *Ibid.*, p. 715.

Contribution to the Chemistry and Pharmacology of the *Digitalis purpurea* Glycosides*

By E. W. McCHESNEY, F. C. NACHOD, M. E. AUERBACH, and F. O. LAQUER

Pure samples of gitoxin, digitoxin, and their aglucones have been prepared. An analytical method has been devised for the determination of the proportion of digitoxin and gitoxin plus gitalin in a mixture. The principle involved depends upon the colors developed by the action of concentrated sulfuric acid on digitoxigenin and gitoxigenin.

IN RECENT years there has been a considerable increase of interest in the cardiac glycosides obtained from *Digitalis purpurea* (foxglove), due chiefly to the work of Cloetta, Windaus, and Jacobs, and their co-workers. Purified glycoside preparations, usually designated as digitoxin, have been available commercially in Europe for many years, and more recently in this country. The digitoxin content of these preparations probably exceeds 90 per cent but, unless especially purified, some of another active *D. purpurea* glycoside, namely gitoxin, is also present.

The pharmacology of gitoxin has never been completely worked out, presumably because sufficient quantities of authentic material have not been available to those interested. The pharmacology of digitoxin has, of course, been quite thoroughly studied. Fromherz and Welsch (1) compared the toxicity of the two substances in frogs and cats. In the former species they found gitoxin to have an activity equivalent to 40 per cent of that of crystalline digitoxin, and in cats, using the Hatcher-dose method (2), they found an activity equivalent to 70 per cent of digitoxin. Cloetta (3*b*) gave the frog and cat toxicity of his "bigitalinum" (apparently identical with gitoxin) as about 35 per cent of that of digitoxin. Chen, Chen, and Anderson (4) reported determinations of the toxicity of a number of cardiac glycosides but found gitoxin too insoluble in alcohol and water to permit a determination of its toxicity. We have not encountered this difficulty.

The reaction of gitoxin with sulfuric acid to produce a brilliant red color is well known, having been observed by Cloetta (3*b*) and others. We felt that it might be possible to develop this qualitative reaction into a quantitative method for the analysis of a mixture of the two glycosides, since with sulfuric acid, pure digitoxin gives only a yellowish brown color. Such a method would be particularly useful in the process of purification of both substances, and for the analysis of commercial preparations which may be mixtures of the two.

* Received December 19, 1947, from the Sterling-Winthrop Research Institute, Rensselaer, N. Y.

DESCRIPTION OF PREPARATIONS USED

Digitoxin.—U. S. P. quality digitoxin was recrystallized several times from 75% methanol, using charcoal for complete decolorization. The crystallizations were repeated until a constant melting point and crystal form were obtained. There is no single definitely established criterion in the literature by which a pure preparation of digitoxin can be identified, but the melting point of 255–257° given by Windaus and Freese (5) seems to be the one most commonly obtained.[1] This, however, depends on the rate of heating, and an impure preparation may give nearly as high a value. In all cases described below the melting point was taken with a Kofler hot stage microscope, using an average temperature rise of about 15°/min., or, alternatively, by heating a bath to about 20° below the expected melting point before immersing the melting-point tube, then increasing the temperature at the rate of 5°/min. All readings recorded are corrected for stem exposure.

Preparation A.—Opaque colorless needles, m. p. (hot stage) 253–255°, alternate method, 238–240°. Chloroform solubility, 1:30 by weight. Elementary analysis: C, 64.35%; H, 8.23%. Calculated: C, 64.38%; H, 8.41%. $[\alpha]_D^{23} = +15 \pm 1°$ (1% in 3:1 chloroform-methanol); in literature $[\alpha]_D^{21} = +21°$ (0.55% in $CHCl_3$) (6). The chloroform solubility (1:92) of the preparation on which the latter value was recorded leaves its authenticity somewhat in doubt.

Preparation B.—Opaque colorless plates and needles. M. p. (hot stage), 253°, alternate method, 236–237°. Chloroform solubility, 1:20. Elementary analysis: C, 64.07%; H, 8.58%. This preparation is evidently not quite pure in spite of its excellent physical characteristics.

Gitoxin.—This substance was prepared from commercial chloroform-insoluble residues by the method of Windaus and Schwarte (7). The crystalline material obtained consisted of oblong rectangular plates or prisms resembling closely those pictured by Stoll (8). M. p. (hot stage), 278°; in literature (3*b*), 282°; alternate method, 263–265°; in literature (9), 266–269°. Chloroform solubility, 1:11,000 by weight (determined colorimetrically on an aliquot of a saturated solution by the method of James, Laquer, and McIntyre (10)). Elementary analysis:

[1] When their preparation was crystallized from aqueous alcohol, it melted at 233–235°.

[2] We attempted to solve the problem of the low solubility of gitoxin by dissolving it in CH_3OH:HCOOH = 1:4. This gave $[\alpha]_D^{23} = +70°$, and for digitoxin A $[\alpha]_D^{23} = +54°$. The rotation of gitoxigenin was not enhanced by this solvent mixture.

C, 62.94%; H, 8.11%; Calculated: C, 63.05%; H, 8.26%. $[\alpha]_D^{24} = +5 \pm 1°$ (1% in pyridine).[3] In literature $[\alpha]_{Hg}^{20} = +3.5°$ (in pyridine) (11).

Digitoxigenin.—This substance was prepared from pure digitoxin as described by Cloetta (3*a*). M. p. by either method, 247–250°; in literature, 251° (13). Analysis: C, 73.86%; H, 9.02%; Calculated: C, 73.76%; H, 9.15%. $[\alpha]_D^{24} = +20 \pm 1°$ (1% in 3:1 chloroform-methanol). In literature, $[\alpha]_D^{17} = +19.1°$ (12).

Gitoxigenin.—Prepared from pure gitoxin according to Windaus and Schwarte (7). M. p. by either method, 222–224°; in literature, 224–225°. Analysis: C, 70.90%; H, 8.72%; Calculated: C, 70.73%; H, 8.74%. $[\alpha]_D^{23} = +35 \pm 1°$ (1% in 3:1 chloroform-methanol). In literature, $[\alpha]_D^{20} = +33.6°$ (in methanol) and $[\alpha]_{Hg}^{20} = +38.5°$ (in methanol) (11).

EXPERIMENTAL

Color Reaction of Gitoxigenin and Digitoxigenin with Sulfuric Acid.—Windaus and Schwarte noted that gitoxigenin gives a red color with ferric chloride in the presence of strong sulfuric acid. For example, if 50 γ of the aglucone are dissolved in 5 cc. of the concentrated acid and 1 drop of 5% ferric chloride solution is added, a beautiful cherry-red color develops within a few seconds. This color reaction does not seem well adapted to colorimetric work since the intensity increases rapidly up to about 5 minutes, and then the red color fades and is replaced by brown. On the other hand, if the ferric chloride is not added, the same color develops slowly, reaches a maximum in about twenty-four hours, and, to the naked eye, remains unchanged for several days. The time factor is thus not critical. Digitoxigenin treated similarly gives at first a pale lemon-yellow color which gradually deepens. The glycosides give similar color reactions, but the aglucones are much better adapted to colorimetric work than are the glycosides since with the latter there is admixed a brown color resulting from the reaction of sulfuric acid on the digitoxose. The absorption spectra of the aglucones in sulfuric acid were studied in a Beckman Spectrophotometer and are shown in Fig. 1.

It may be seen from the figure that the different behavior of the two aglucones provides a basis for the analysis of a mixture. At about 420 mμ there is an isosbestic point, while at 525 mμ the absorption coefficient of gitoxigenin is about 10 times that of digitoxigenin. (In a photoelectric colorimeter, however, using a 520 filter, the ratio of optical densities is not so favorable, being rather of the order of 5–7 times.) The curves show, furthermore, that after twenty-four hours no rapid change is taking place in the gitoxigenin color; therefore approximately this interval was used in the colorimetric work.

It is of interest to note at this point that metallic catalysts other than iron may be used for more rapid color development. If a drop of dilute copper sulfate is added, the gitoxigenin solutions develop a maximum color in about four hours and fade somewhat after twenty-four hours. The red color is about 50% more intense at the maximum than is ever developed in the absence of copper, while with digitoxigenin the color intensity is essentially unchanged by the addition of copper. However, the use of this technique has given results which are no more reliable than those obtained without copper, and the idea has not been pursued further. Vanadic and mercuric salts also serve excellently as catalysts for this color reaction, suggesting that it is oxidative in nature.

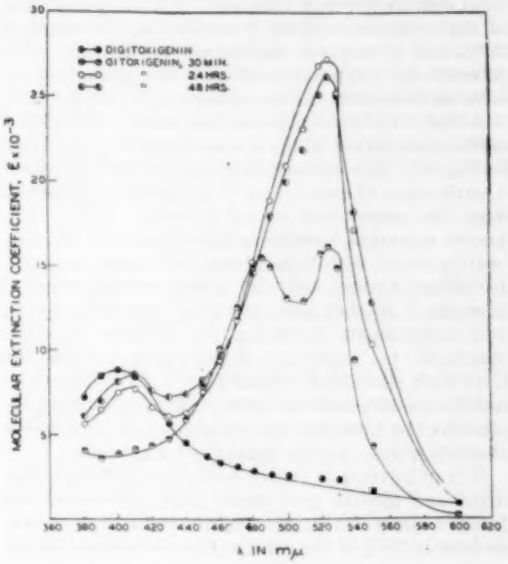

Figure 1.

ANALYTICAL PROCEDURE

Preparation of Samples and Standards.—Twenty milligrams of the sample are hydrolyzed by refluxing for forty-five minutes with 10 cc. of 0.13 *N* HCl in 50% ethanol, as directed by Windaus and Schwarte. The hydrolyzate is transferred to a separatory funnel with the aid of a little alcohol, and three volumes of water are added, followed by enough *N* NaOH to neutralize the HCl. The aglucone is extracted by shaking twice with 25 cc. chloroform, and the chloroform solution is made up to 100 cc. with alcohol. This solution contains almost exactly 100 γ of aglucone per cc., but the quantity is not critical except in the standards used for calibration.

Portions of 1 cc. of the aglucone solution are evaporated to dryness in scrupulously clean test tubes, and 5 cc. of concentrated sulfuric acid are added. Extreme accuracy in measurement is not necessary. The tubes are allowed to stand in a dry place (in bell jar or desiccator) for twenty to twenty-two hours. The color is then read in a photoelectric colorimeter against a sulfuric acid blank, using both 420 and 520 filters. An instrument which gives readings directly proportional to the optical density is preferable. The 520 reading is divided by the 420 reading and the ratio recorded. If a series is properly set up all of the 420 readings will be identical within ±5%. Any reading which is very high suggests contamination with organic matter and should be repeated.

Standards consist of hydrolyzed pure gitoxin and digitoxin treated as previously described.[3,4] A series for calibration is most conveniently set up as follows: In a series of 6 test tubes labeled 100, 80, 60, 40, 20, and 0 measure the following quantities of hydrolyzed digitoxin, respectively: 1.0, 0.8, 0.6, 0.4, 0.2 0. cc. Then measure in the following quantities of hydrolyzed gitoxin, respectively: 0, 0.2, 0.4, 0.6, 0.8, 1.0 cc. Each tube then contains a total of almost exactly 100 γ of aglucone, with the percentage of digitoxigenin ranging from 100 to 0 in steps of 20%, and gitoxigenin making up the balance. The solvents are evaporated off and the procedure for color development outlined above is followed.

About five repetitions of this series will give a calibration curve[5] which is a straight line, as shown in Fig. 2. The individual points plotted represent a sixth series of tubes, and their standard deviation from the established curves is about ±2%. Unknown materials have been plotted against this calibration curve with typical results as given in Table I. When known mixtures have been hydrolyzed (samples 1–3) they have given the expected results, and experiments involving the addition of pure standards to materials of unknown composition have been successful (samples 9, 11–13). Since the results are obtained as ratios, they must be multiplied by the total glycoside content (10) to give the absolute values for the individual glycosides.

It is of interest to record here that, although digitoxin and gitoxin give equal color values by the James-Laquer-McIntyre method, crystalline gitoxin gives only 28% of the color value of digitoxin (Preparation A) by the Anderson-Chen method (13). Similarly, by the latter procedure, digitoxigenin gives 195%, and gitoxigenin 37%, as much color as digitoxin (A). The color reaction has been attributed to the active methylene hydrogen of the lactone ring (14), but for some reason this atom is less active in gitoxigenin and gitoxin. The difference is not solely one of the reaction time since the color intensity with gitoxin never equals that of digitoxin. It has also been noted (15) that gitoxin is less active when the analysis is made by the Bell-Krantz method (16).

It must be concluded, therefore, that the James *et al.* method is a more accurate index of total glycoside content than the Anderson-Chen, while the latter method is more nearly specific for digitoxin

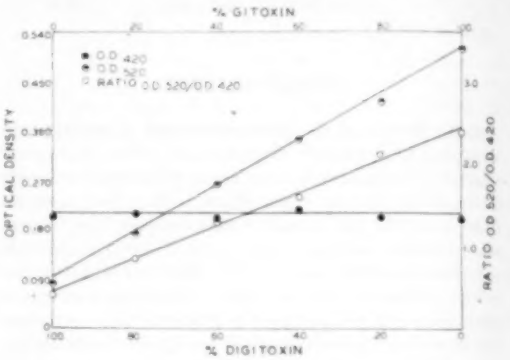

Figure 2.

TABLE I.—ANALYSIS OF SOME TYPICAL GLYCOSIDE PREPARATIONS FOR GITOXIN/DIGITOXIN DISTRIBUTION

Sample	Composition	No. of Trials	$\frac{D_{520}}{D_{420}}$, Av.	% Gitoxin[a] ± S. E.	% Gitoxin Calculated
1	5 mg. Digitoxin (A) + 15 mg. Gitoxin	4	1.91	72.8 ± 1.3	75
2	10 mg. Digitoxin (A) + 10 mg. Gitoxin	9	1.46	50.2 ± 3.7	50
3	15 mg. Digitoxin (A) + 5 mg. Gitoxin	5	0.93	24.2 ± 1.6	25
4	20 mg. Digitoxin (B)	5	0.49	2.2 ± 0.9	...
5	U. S. P. Digitoxin Reference Standard	6	0.51	3.2 ± 1.4	...
6	Commercial U. S. P. XIII Digitoxin	5	0.67	10.6 ± 0.5	...
7	Commercial U. S. P. XIII Digitoxin	5	0.43	−0.6 ± 0.8	...
8	Crude chloroform insoluble residues	6	1.70	62.0 ± 1.8	...
9	10 mg. Sample 8 + 10 mg. Digitoxin Std. hydrolyzed together	6	1.08	31.0 ± 0.96	31[b]
10	Partially purified Gitoxin	7	2.12	83.5 ± 2.2	...
11	0.5 cc. from Sample 8 + 0.5 cc. Digitoxin Std.	5	1.08	31.0 ± 1.7	31[b]
12	0.5 cc. Sample 11 + 0.5 cc. Digitoxin Std.	5	1.30	42.5 ± 0.9	41.8[b]
13	0.5 cc. Sample 6 + 0.5 cc. Gitoxin Std.	5	1.57	55.2 ± 2.3	55.3[b]

[a] And/or *Gitalinum cristallisatum*. Digitoxin makes up the remainder of 100%.
[b] Calculated assuming that the values already obtained for the unknown portions of these samples are correct.

[3] Samples of digitoxin and gitoxin may be obtained from the Special Chemicals Division of Winthrop-Stearns, Inc., 1450 Broadway., New York, N. Y.

[4] If pure aglucones are used as standards the calibration curve has a somewhat steeper slope, chiefly because the readings at 420 mμ are low and inconsistent. This could have two possible interpretations: (1) the digitoxin and gitoxin preparations used as standards are not entirely pure, or (2) a trace of impurity in the hydrolyzates affects the color development. The second explanation seems more plausible since the calibration curve established with the available samples of standards gives more reasonable results for all of the preparations. For example, against the aglucone standards, the U. S. P. reference standard would analyze 8% gitoxin and all the other results would be shifted toward the 50% mark.

[5] Different samples of sulfuric acid may give different calibration curves depending on their content of heavy metals.

than the former. For example, a preparation containing 70% digitoxin, 30% gitoxin would assay about as follows (in terms of digitoxin): James *et al.* method, 100%; Anderson-Chen method, 78%; cat method, 96%. (NOTE: it is assumed that the cat potency of gitoxin is 90% of that of digitoxin; see below.)

Interfering Substances.—Other constituents of *D. purpurea* such as pigments, tannins, chlorophyll, lipids, etc., are usually well eliminated by the time a preparation has reached a total glycoside content of 95% or more, which probably should be the case before the present method is applied. The saponins

(digitonin, gitonin, and tigonin) might still be present in minute amounts.[6] These substances are difficult to hydrolyze under the conditions prescribed for the glycosides, and they yield different genins. Gitogenin, sometimes a contaminant, gives a pale yellow color with sulfuric acid, but of a lesser intensity than that of digitoxigenin (19). It is unlikely, therefore, that these would offer any serious interference. If, however, the substance described by Cloetta (3*b*) under the name *Gitalinum cristallisatum* is actually present in the leaves of *D. purpurea*, it would affect the results. It is believed to be a glycoside consisting of 2 molecules of digitoxose and a monohydrate of gitoxigenin. The per cent of digitoxin in a given preparation would, therefore, be as determined from the graph, but what is designated as "per cent gitoxin" would presumably represent gitoxin plus *Gitalinum*. Some entirely different principle would have to be applied to the analysis of a mixture of the latter two substances if their color reactions with sulfuric acid are actually the same.

Ultraviolet Absorption.—As would be expected, the glycosides show chiefly end-absorption. However, when examined in ethanol solution over the range 212–265 mμ, gitoxin shows a definite maximum at 219 mμ with $E^{1\%}_{1\,cm.} = 191$, while digitoxin shows no definite maximum but at 215 mμ, $E^{1\%}_{1\,cm.} = 263$.

Toxicity of Gitoxin.—The crystalline glycoside was ground to a fine powder and 25 mg. were dissolved in 73 cc. of 95 % ethanol by warming to 50° and shaking occasionally until solution was complete. Water was carefully added to a volume of 100 cc.; the solution remained clear. This was then diluted in the ratio 4/35 with 0.9% NaCl in 20% ethanol, again without precipitation. When this solution was administered to cats the average lethal dose was 15.5 ± 0.57 cc. or 0.443 ± 0.014 mg. of gitoxin. The U. S. P. Digitoxin Reference Standard, tested concomitantly, gave an average lethal dose of 0.375 ± 0.015 mg.; therefore, gitoxin would appear to be 83% as toxic as digitoxin. LaWall and Harrisson, Consultants, of Philadelphia, tested the same sample of gitoxin and reported a toxicity equivalent to 1859 U. S. P. Digitalis Units per Gm., or about 95% of the 1900–2000 units found for the average U. S. P. XIII digitoxin.

DISCUSSION

Gitoxin has been found in this work to have a biological activity closely approaching that of digitoxin. This is logical since the view is generally accepted that the lactone ring is responsible for the toxicity (17). There would prove to be little justification for an expensive process of purification to remove this "impurity," in the event that the pharmacological and therapeutic properties of digitoxin and gitoxin are otherwise similar.

The analytical procedure described in this paper gives an indication of the relative amounts of digitoxin and gitoxin (and/or *Gitalinum*) present in the simple case where the preparation consists almost entirely of glycosides. It might conceivably have some application to the analysis of the lanatosides (Digilanid B) since digoxigenin, as well as digitoxigenin, does not give a red color with sulfuric acid (18).

Purified glycosides which meet present U. S. P. requirements for digitoxin may contain an appreciable amount of gitoxin (and/or *Gitalinum*). The criterion of chloroform solubility prescribed in U. S. P. XIII does not guarantee the absence of gitoxin.

The authors gratefully acknowledge the data contributed to this paper by two members of the staff of Winthrop-Stearns, Inc. Dr. H. B. Corbitt conducted the analyses by the Anderson-Chen method, and Mr. K. D. Sprague determined the lethal dose of gitoxin for cats.

SUMMARY

1. Analytically pure samples of gitoxin, digitoxin, and their aglucones have been prepared.

2. Gitoxin has been found to have a toxicity in cats only slightly less than that of digitoxin.

3. Digitoxigenin and gitoxigenin may be distinguished by means of the colors they develop in concentrated sulfuric acid. This reaction has been developed into an analytical method which gives the proportion of digitoxin and gitoxin plus gitalin in a mixture. This method should be of value in following purification processes and in the analysis of commercial preparations.

REFERENCES

(1) Fromherz, K., and Welsch, A., *Arch. expil. Path. Pharmakol.*, **161**, 266(1931).
(2) Magnus, V., and van Wijngaarden, de Lind, *ibid.*, **114**, 21(1926).
(3) Cloetta, M., *ibid.*, (*a*) **88**, 113(1920); (*b*) **112**, 261 (1926).
(4) Chen, K. K., Chen, A. L., and Anderson, R. C., THIS JOURNAL, **25**, 579(1936).
(5) Windaus, A., and Freese, C., *Ber.*, **58B**, 2503(1925).
(6) Küssner, W., *Arch. Pharm.*, **279**, 41(1941).
(7) Windaus, A., and Schwarte, G., Ber., **58B**, 1515 (1925).
(8) Stoll, A., "The Cardiac Glycosides," Heffer and Sons, Ltd., Cambridge, 1937, p. 70.
(9) Windaus, A., Westphal, K., and Stein, G., *Ber.*, **61B**, 1847(1928).
(10) James, A. E., Laquer, F. O., and McIntyre, J. D., THIS JOURNAL, **36**, 1(1947).
(11) Smith, S., *J. Chem. Soc.*, **1931**, 23.
(12) Windaus, A., and Stein, G., Ber., **61B**, 2436(1928).
(13) Anderson, R. C., and Chen., K. K., THIS JOURNAL, **35**, 353(1946).
(14) Raymond, W. D., *Analyst*, **64**, 113(1939).
(15) Krantz, J. C., Jr., and Bell, F. K., *Federation Proc.*, **6**, 346 (1947).
(16) Bell, F. K., and Krantz, J. C., Jr., *J. Pharmacol. Expil. Therap.*, **83**, 213(1945).
(17) Windaus, A., Bohne, A., and Schwieger, A., *Ber.*, **57B**, 1388(1924).
(18) Smith, S., *J. Chem. Soc.*, **1930**, 508.
(19) Iwamoto, H. K., Bell, F. K., Laquer, F. O., Carr, C. J., and Krantz, J. C., Jr., *J. Pharmacol. Expil. Therap.*, **91**, 130(1947).

[6] U. S. P. XIII specifications eliminate these substances qualitatively.

Effect of Particle Size, Type of Base, and a Wetting Agent on Three Antiseptic Ointments*

By LEE H. MACDONALD and R. E. HIMELICK†

In the official ointments of ammoniated mercury, yellow mercuric oxide, and calomel, a reduction in the particle size of the mercurial usually contributes to increased antiseptic activity, as measured by the agar plate method. Likewise, the addition of the wetting agent dodecylamine hydrochloride to the ointments causes a moderate increase in the zones of inhibition. Substituting an emulsion base (containing cetyl alcohol, Carbowax, sodium lauryl sulfate, and water) for the official bases greatly increases the inhibition zones in all cases.

MANY STUDIES have been made to determine the relative effectiveness of grease-type and emulsion-type bases for antiseptic ointments. Gershenfeld and Brillhart (1), for example, found that ointments of ammoniated mercury, phenol, and mercuric chloride produced greater bacteriostatic action when the medicaments were incorporated into water-miscible bases. Fiero and Loomis (2) have reported the antiseptic activity of several official ointments to be enhanced by substituting emulsified bases for the official bases.

In the field of sulfonamide ointments, emulsion bases have been particularly recommended. Howard (3) determined that emulsion bases yielded high levels of sulfonamides to a surrounding aqueous medium, while grease and cold cream bases yielded little. Bases containing at least 50 per cent water were found by Aldrich and DeKay (4) to give optimum activity in sulfonamide ointments. They also observed increased penetrative power when surface-active agents were added.

The effect of the particle size of the active ingredients in antiseptic ointments has also been studied. Vichers, Snyder, and Gathercoal (5) found that a calomel ointment prepared from a specially processed colloidal calomel showed a much higher antiseptic power than one made from U. S. P. calomel, and they postulated that the decreased particle size might account for its increased effectiveness. Other explanations for this phenomenon have been offered, however (6). Fleming and Wolf (7) made a series of calomel ointments, varying the particle size of the calomel, and tested them as prophylactic agents for experimental syphilis in rabbits. They found an ointment made with 100 micron calomel to be relatively ineffective, with effectiveness increasing as particle size was reduced. Laug, *et al.* (8) also observed that reducing the particle size of calomel increased the penetration of mercury. They found that surface-active agents increased skin penetration when incorporated into an animal fat or emulsion base, but detected no effect when the agent was incorporated into a mineral base.

In this study we have attempted to evaluate the effect of (*a*) type of base, (*b*) particle size, and (*c*) the surface-active agent dodecylamine hydrochloride on the antiseptic activity of three commonly used mercury-containing ointments: ammoniated mercury, calomel, and yellow mercuric oxide.

EXPERIMENTAL

A series of ammoniated mercury ointments was prepared and tested for antiseptic activity by the agar plate method.[1] Formulas and zones of inhibition are indicated in Table I.

Results obtained with a series of yellow mercuric oxide ointments are indicated in Table II.

Two series of calomel ointments were prepared and tested, one at the N. F. strength of 30% and another at the 10% level. The latter concentration

TABLE I.—AMMONIATED MERCURY OINTMENTS

Ammoniated Mercury, %	Av. Particle Size in μ	Base	Dodecylamine Hydrochloride, %	Zone of Inhibition, Mm.
5	8	U. S. P. XIII	0	5
5	8	U. S. P. XIII	0.1	6
5	8	Emulsion[a]	0	9
5	3	U. S. P. XIII	0	5
5	3	U. S. P. XIII	0.1	6
5	3	Emulsion	0	9
0	.	U. S. P. XIII	0	0
0	.	U. S. P. XIII	0.1	0
0	.	Emulsion[a]	0	0

[a] The emulsion base used throughout this study contained 25% cetyl alcohol, 25% Carbowax 1500, 1% sodium lauryl sulfate, and 49% water.

[1] A 20-cc. portion of meat infusion agar is melted and cooled to 45°. To this is added 0.2 cc. of an eighteen-hour broth culture of *Staphylococcus aureus* 209 P. This inoculated agar is then poured into a sterile Petri plate and allowed to harden, after which 0.5 Gm. of the ointment is placed in the center of the agar. The plates are incubated at 37°, and read after forty-eight hours. Inhibition zones are measured from the edge of the ointment to the edge of the zone of complete inhibition.

* Received Dec. 10, 1947, from the Research Division of The Upjohn Company, Kalamazoo, Mich.

† We wish to acknowledge the assistance of the Bacteriology Research Department of The Upjohn Company in compiling the bacteriological data for this study.

was chosen so that comparisons might be made with the colloidal calomel of Vichers, *et al.* (5), which does not lend itself well to incorporation into a 30% ointment. Formulas and zones of inhibition obtained with both series are shown in Table III.

TABLE II.—YELLOW MERCURIC OXIDE OINTMENTS

Mercuric Oxide %	Av. Particle Size in μ	Base	Dodecylamine Hydrochloride, %	Zone of Inhibition, Mm.
1	10	U. S. P. XIII	0	1
1	10	U. S. P. XIII	0.1	6
1	10	Emulsion	0	10
1	1.5	U. S. P. XIII	0	4
1	1.5	U. S. P. XIII	0.1	7
1	1.5	Emulsion	0	10
0	..	U. S. P. XIII	0	0
0	..	U. S. P. XIII	0.1	0
0	..	Emulsion	0	0

antiseptic efficacy of the grease-base ointments, as measured by the plate method.

SUMMARY

A series of ammoniated mercury, yellow mercuric oxide, and calomel ointments has been prepared and tested for antiseptic activity by the agar plate method. The effect of particle size, type of base, and the addition of a wetting agent on the size of the inhibition zone has been studied.

Reduction of particle size and the addition of the surface-active agent dodecylamine hydrochloride usually contributed to the production of an increased zone of inhibition in the grease-base ointments.

Substitution of an emulsion base for the grease base resulted in an increased inhibition zone in all cases.

TABLE III.—CALOMEL OINTMENTS

Calomel, %	Av. Particle Size in μ	Base	Dodecylamine Hydrochloride, %	Zone of Inhibition, Mm.
30	5	N. F. VIII	0	1.5
30	5	N. F. VIII	0.1	1.5
30	5	Emulsion	0	3.5
30	2	N. F. VIII	0	2
30	2	N. F. VIII	0.1	3
30	2	Emulsion	0	5
10	5	N. F. VIII	0	0
10	5	N. F. VIII	0.1	0
10	5	Emulsion	0	3
10	2	N. F. VIII	0	0
10	2	N. F. VIII	0.1	1.5
10	2	Emulsion	0	5
10	Colloidal[a]	N. F. VIII	0	7.5
10	Colloidal	N. F. VIII	0.1	8.5
10	Colloidal	Emulsion	0	10
0	....	N. F. VIII	0	0
0	....	N. F. VIII	0.1	0
0	....	Emulsion	0	0

[a] Less than 1 μ.

DISCUSSION

From these results it appears that ointments of ammoniated mercury, yellow mercuric oxide, and calomel are least effective in their present official bases, if the agar plate test can be taken as a criterion of their efficacy. In all cases the use of an emulsion-type base gave ointments showing greater zones of inhibition.

In most instances reduction in particle size of the mercury compound and the addition of 0.1% dodecylamine hydrochloride each contributed to the

REFERENCES

(1) Gershenfeld, L., and Brillhart, R. E., *Am. J. Pharm.*, **111**, 430(1939).
(2) Fiero, G. W., and Loomis, T. A., THIS JOURNAL, *Pract. Pharm. Ed.*, **3**, 170(1942).
(3) Howard, R. W., *New England J. Med.*, **232**, 698(1945).
(4) Aldrich, D. F., and DeKay, H. G., THIS JOURNAL, **34**, 17(1945).
(5) Vichers, E. E., Snyder, R. K., and Gathercoal, E. N., *ibid.*, **26**, 1241(1937).
(6) Schiller, F. W., *Am. J. Pharm.*, **110**, 289(1938).
(7) Fleming, W. L., and Wolf, M. H., *Am. J. Syphilis, Gonorrhea, Venereal Diseases*, **30**, 47(1946).
(8) Laug, E. P., Vos, E. A., Kunze, F. M., and Umberger, E. J., *J. Pharmacol. Expll. Therap.*, **89**, 52(1947).

Stability Study of Penicillin Ointment*

By S. H. CULTER†

A number of penicillin ointments using various types of bases with sodium, calcium, and potassium salts of penicillin of varying degrees of potency were prepared. These ointments were stored at room and refrigerator temperatures and assayed from time to time to determine the stability of the penicillin. It was found that the impure sodium penicillin ointment was quite unstable as compared to the calcium salt of similar purity. The crystalline sodium and potassium salts of penicillin were equal to, if not superior to, the calcium salt as far as stability was concerned. The presence of sulfathiazole, sulfadiazine, benzocaine, epinephrine, and phenacaine does not appreciably affect the stability of the sodium, calcium, or potassium penicillin in the ointment bases tried in these experiments. The presence of water, Carbowax, zinc stearate, or dried aluminum hydroxide gel greatly hastens the decomposition of penicillin under the conditions of the experiments.

When crude sodium penicillin first became available in sufficient quantities for various experimental purposes in 1944 it was decided to prepare an ointment of this antibiotic suitable for topical application as well as one for ophthalmic use. It was realized, of course, that the chief problem involved would be one of selecting a base in which the penicillin then available was stable. This was especially important since the resulting product was intended for commercial distribution, which meant that it should lose not more than a relatively small percentage of its potency in a year at refrigerator temperature. Nearly all penicillin ointments and creams mentioned in the literature (1–8) at that time were intended for extemporaneous use because of their relative instability.

Later, when the impure calcium salt of penicillin became available in sufficient quantities for experimental purposes, it was incorporated into bases similar to those used for the impure sodium salt. It was also combined in ointment form with other medicaments such as sulfathiazole, sulfadiazine, phenacaine hydrochloride, benzocaine, and epinephrine.

When the crystalline sodium and potassium salts of penicillin were available they were also prepared in ointment form alone and in combinations with other medicaments.

The ointments prepared for this investigation were made under ordinary laboratory conditions of temperature and humidity. The samples were stored in collapsible tin tubes at room temperature and most of them also at refrigerator temperature. They were assayed by the agar cup-plate method at the time of preparation and at various intervals thereafter. Those in a water-soluble or water-miscible base were first dissolved or shaken with water and an aliquot taken for testing, while those in a grease base were dissolved in ether and the penicillin extracted with a phosphate buffer solution.

EXPERIMENTAL

Stability of Ointments Made with Impure Sodium Penicillin.—Since it is generally accepted that water-soluble constituents in ointment form diffuse more readily from a water-miscible base than from a grease base, it was decided to incorporate crude sodium penicillin into this type of base. Thus one sample was prepared using Carbowax 1500 alone and another using a water-miscible base containing a high percentage of water. Several samples with an anhydrous base containing petrolatum as the chief ingredient were also prepared for comparison in stability studies. The results are shown in Table I.

From the data in Table I it can readily be seen that crude sodium penicillin is much more stable in a nonaqueous base or in one which does not contain Carbowax 1500. However, it is not sufficiently stable in these bases for commercial consideration, but perhaps would be suitable for extemporaneous use.

Stability of Ointments Made with Impure Calcium Penicillin.—When the impure calcium salt of penicillin became available for experimental use, it was tried alone in some of the bases given in Table I, as well as in combinations with other medicaments. The results of these experiments are given in Table II. The oils and petrolatum marked "dried" in this table were heated to about 120° under vacuum for at least two hours and allowed to cool before using to remove any traces of moisture which may have been present.

It is readily seen from the stability data in Table II that calcium penicillin is much more stable in the ointment bases used than the impure sodium salt. Even in the aqueous base or in Carbowax 1500 calcium penicillin is sufficiently stable for prescription use, especially if the ointment is kept at refrigerator temperature. This observation has also been confirmed by others (2–9). It is also specified in the U. S. P. XIII that Penicillin Ointment shall contain only the calcium penicillin.

* Received April 26, 1948, from the Research Laboratories of The Upjohn Company, Kalamazoo, Mich.

† The author wishes to thank Dr. H. G. Kolloff and Mr. R. E. Himelick for their advice and suggestions in this work and the Bacteriological Research Department of The Upjohn Company for the bacteriological data and numerous penicillin assay determinations necessitated by the stability studies.

TABLE I.—STABILITY OF OINTMENTS CONTAINING IMPURE SODIUM PENICILLIN

Formula		Time	Per Cent of Theoretical Penicillin Potency: Room Temp.	Refrig. Temp.
Penicillin Sodium (400 U./Mg.)	0.1%	0	96	
Carbowax 1500	*q. s.*	4 days	30	
		8 days	10	
Penicillin Sodium (400 U./Mg.)	0.1%			
Carbowax 1500	25.0%	0	60	
Stenol	25.0%	11 days	15	30
Sodium lauryl sulfate	1.0%	2 mo.		11
Water	*q. s.*			
Penicillin Sodium (400 U./Mg.)	0.1%	0	75	
Wool fat, anhydrous	5.0%	3 wks.	35	
White Petrolatum	*q. s.*			
Penicillin Sodium (400 U./Mg.)	0.1%	0	75	
Aquaphor	5.0%	6 wks.	45	
White Petrolatum	*q. s.*			
Penicillin Sodium (400 U./Mg.)	0.1%	0	87	
Falba Absorption Base	5.0%	2 wks.	42	
White Petrolatum	*q. s.*			
Penicillin Sodium (400 U./Mg.)	0.1%	0	89	
Peanut Oil	5.0%	2 wks.	36	
White Petrolatum	*q. s.*			
Penicillin Sodium (400 U./Mg.)	0.1%	0	85	
Corn Oil	5.0%	2 wks.	21	
White Petrolatum	*q. s.*			
Penicillin Sodium (400 U./Mg.)	0.1%	0	90	
Cod Liver Oil	5.0%	2 wks.	39	
White Petrolatum	*q. s.*			
Penicillin Sodium (400 U./Mg.)	0.1%	0	100	
Cottonseed Oil	5.0%	2 wks.	46	
White Petrolatum	*q. s.*			

TABLE II.—STABILITY OF OINTMENTS CONTAINING IMPURE CALCIUM PENICILLIN

Formula		Time	Per Cent of Theoretical Penicillin Potency: Room Temp.	Refrig. Temp.
Penicillin Calcium (650 U./Mg.)	0.1%	0	100	
Carbowax 1500 (dried)	*q. s.*	2 wks.	7	50
		1 mo.	..	19
Penicillin Calcium (650 U./Mg.)	0.12%	0	93	93
Carbowax 1500	25.0%	11 days	20	71
Stenol	25.0%	18 days	7	54
Sodium Lauryl Sulfate	1.0%	2 mo.	..	19
Water	*q. s.*			
Penicillin Calcium (500 U./Mg.)	0.1%	0	94	
Cottonseed Oil	5.0%	4 mo.	87	
White Petrolatum	*q. s.*	6 mo.	75	
		1 yr.	62	
		19 mo.	67[a]	
Penicillin Calcium (500 U./Mg.)	0.1%	0	100	
Mineral Oil	5.0%	1 mo.	100	
White Petrolatum	*q. s.*	8 mo.	78	
		15 mo.	60	
Penicillin Calcium (650 U./Mg.)	0.1%	0	100	
Sulfathiazole	5.0%	5 mo.	88	90
Cottonseed Oil (dried)	5.0%	9 mo.	88	100[a]
White Petrolatum (dried)	*q. s.*	15 mo.	60	71
Penicillin Calcium (650 U./Mg.)	0.12%	0	100	
Benzocaine	5.0%	6 mo.	89	95
Cottonseed Oil (dried)	5.0%	1 yr.	84	74
White Petrolatum (dried)	*q. s.*	15 mo.	72	81[a]
		2 yr.	63	68[a]

(*Cont'd on p. 372*)

TABLE II.—STABILITY OF OINTMENTS CONTAINING IMPURE CALCIUM PENICILLIN. (*Cont'd from page 371*)

Formula		Time	Per Cent of Theoretical Penicillin Potency: Room Temp.	Refrig. Temp.
Penicillin Calcium (650 U./Mg.)	0.1%	0	100	
Epinephrine	0.0125%	6 mo.	86	100
Boric Acid	0.0125%	20 mo.	71	75
Cottonseed Oil (dried)	5.0%	2 yr.	71	75
White Petrolatum (dried)	*q. s.*			
Penicillin Calcium (650 U./Mg.)	0.1%	0	100	
Epinephrine	0.0125%	6 mo.	65	96
Boric Acid	0.0125%	20 mo.	36	82
Cottonseed Oil (dried)	40.0%	26 mo.	..	65
White Petrolatum (dried)	*q. s.*			
Penicillin Calcium (650 U./Mg.)	0.12%	0	100	
Phenacaine Hydrochloride	0.75%	6 mo.	61	89
Cottonseed Oil (dried)	5.0%	1 yr.	65	70
White Petrolatum (dried)	*q. s.*	15 mo.	75[a]	89[a]
		2 yr.	47	71
Penicillin Calcium (650 U./Mg.)	0.042%	0	100	
Sulfadiazine	5.0%	6 mo.	97	100
Cottonseed Oil (dried)	40.0%	18 mo.	60	100
White Petrolatum (dried)	*q. s.*			
Penicillin Calcium (650 U./Mg.)	0.1%	0	100	
Peanut Oil (dried)	5.0%	6 mo.	79	80
White Petrolatum (dried)	*q. s.*	16 mo.	61	77
		2 yr.	35	92[a]
Penicillin Calcium (650 U./Mg.)	0.05%	0	100	
Peanut Oil (dried)	40.0%	6 mo.	82	85
White Petrolatum (dried)	*q. s.*	20 mo.	70	94[a]
		2 yr.	35	70

[a] These inconsistencies in assay results may be explained on the basis of uneven distribution of the penicillin in the base or the inherent difficulties in the assay procedure.

The low percentage of oil in the above formulas was included chiefly to aid in the incorporation and uniform distribution of the penicillin as well as other medicaments in the petrolatum. By increasing the percentage of the oil in the base the consistency can be adjusted as desired. Thus the samples containing 40% oil were much thinner than those containing 5% and were intended for ophthalmic purposes.

The inclusion of sulfathiazole, sulfadiazine, epinephrine, phenacaine, and benzocaine did not materially influence the stability of the calcium penicillin in the ointment bases tested.

Temperature of storage, with the exception of the ointment with the aqueous base, did not greatly influence the stability of the calcium penicillin although the assay results of the samples kept in the refrigerator were usually slightly higher than those kept at room temperature.

Stability of Ointments Made with Crystalline Penicillin.—When the crystalline sodium and potassium salts of penicillin became available these were incorporated into some of the ointment bases described above and stored at room and refrigerator temperatures as before for stability studies. The results are summarized in Table III.

From the stability data on the crystalline sodium and potassium salts of penicillin in the ointments listed in Table III it is quite evident that they are equal if not superior to the impure calcium penicillin in the same or similar ointment bases. However, for a fair comparison a calcium salt of similar potency should also be tested. In this connection it is also interesting to observe the great difference in stability of the pure crystalline sodium penicillin as compared to that of the relatively impure sodium salt in ointment form as shown in Table I.

Further Studies on Penicillin Ointments with Water-Miscible Bases.—Since the first experiments with penicillin in bases containing water or Carbowax 1500 showed a very rapid rate of deterioration of the medicament, it was felt that other bases of the water-miscible type should be tried, especially since this kind of base would be much more desirable than the grease type.

As most of the solids suitable for such a base are too firm to be used as such, it was necessary to find a nonaqueous liquid to give the base an ointment-like consistency. Triacetin was found to be suitable for this purpose. It is somewhat miscible with water and penicillin calcium was found to be fairly stable when suspended in it, as shown in Table IV. Consequently several penicillin ointments were prepared containing triacetin and other ingredients to give a base of suitable consistency.

The formulas and results of stability tests on these preparations are given in Table IV. From the results of penicillin stability tests in this table it can be seen that none of these ointments is as stable as those in the petrolatum-type base even when calcium penicillin of fairly high potency or the crystalline sodium or potassium salts are used.

The presence of zinc stearate and dried aluminum hydroxide gel are greatly detrimental to the stability of the penicillin. On the other hand, corn starch and propylene glycol distearate do not hasten the deterioration rate of penicillin. Calcium gluconate is intermediate in this respect.

TABLE III.—STABILITY OF OINTMENTS CONTAINING CRYSTALLINE PENICILLIN

Formula		Time	Per Cent of Theoretical Penicillin Potency: Room Temp.	Refrig. Temp.
Penicillin Sod. Cryst. (1620 U./Mg.)	0.031%	0	99	
Cottonseed Oil (dried)	5.0%	6 mo.	84	83
White Petrolatum (dried)	q. s.	1 yr.	84	84
Penicillin Pot. Cryst. (1583 U./Mg.)	0.032%	0	97	
Cottonseed Oil (dried)	5.0%	6 mo.	73	73
White Petrolatum (dried)	q. s.	1 yr.	70	72
Penicillin Sod. Cryst. (1620 U./Mg.)	0.031%	0	100	
Sulfathiazole	5.0%	6 mo.	98	99
Cottonseed Oil (dried)	5.0%	9 mo.	72	84
White Petrolatum (dried)	q. s.	1 yr.	76	84
		17 mo.	95[a]	100[a]
Penicillin Pot. Cryst. (1583 U./Mg.)	0.032%	0	100	
Sulfathiazole	5.0%	6 mo.	83	98
Cottonseed Oil (dried)	5.0%	9 mo.	76	78
White Petrolatum (dried)	q. s.	1 yr.	76	82
		17 mo.	88[a]	94[a]
Penicillin Sod. Cryst. (1620 U./Mg.)	0.031%	0	100	
Sulfadiazine	5.0%	6 mo.	96	99
Cottonseed Oil (dried)	5.0%	9 mo.	92	98
White Petrolatum (dried)	q. s.	1 yr.	92	87
		17 mo.	94	100[a]
Penicillin Pot. Cryst. (1583 U./Mg.)	0.032%	0	89	
Sulfadiazine	5.0%	9 mo.	84	90
Cottonseed Oil (dried)	5.0%	1 yr.	82	90
White Petrolatum (dried)	q. s.	16 mo.	72	81
Penicillin Sod. Cryst. (1620 U./Mg.)	0.031%	0	95	
Benzocaine	5.0%	6 mo.	94	93
Cottonseed Oil (dried)	5.0%	9 mo.	93	96
White Petrolatum (dried)	q. s.	1 yr.	74	79
Penicillin Pot. Cryst. (1583 U./Mg.)	0.032%	0	100	
Benzocaine	5.0%	6 mo.	97	100
Cottonseed Oil (dried)	5.0%	9 mo.	98	100
White Petrolatum (dried)	q. s.	1 yr.	76	84

[a] These inconsistencies in assay results may be explained on the basis of uneven distribution of the penicillin in the base or the inherent difficulties in the assay procedure.

TABLE IV.—STABILITY OF OINTMENTS OF PENICILLIN IN OTHER WATER-MISCIBLE BASES

Formula		Time	Per Cent of Theoretical Penicillin Potency: Room Temp.	Refrig. Temp.
Penicillin Calcium (845 U./Mg.)	0.1%	0	100	
Triacetin	q. s.	3 mo.	94	
		4 mo.	91	
		7 mo.	37	
Penicillin Calcium (845 U./Mg.)	0.1%	0	98	
Corn Starch (dried)	60%	3 mo.	84	
Triacetin (dried)	q. s.	6 mo.	57	
		12 mo.	24	
Penicillin Calcium (1097 U./Mg.)	0.05%	1 mo.	67	
Corn Starch (dried)	60%	3 mo.	22	54
Carbowax 4000	8%			
Triacetin (dried)	q. s.			
Penicillin Calcium (1097 U./Mg.)	0.05%			
Zinc Stearate	21.0%	0	No activity	
Triacetin (dried)	q. s.			
Penicillin Calcium (1097 U./Mg.)	0.05%			
Dried Aluminum Hydroxide Gel	26.5%	0	No activity	
Triacetin (dried)	q. s.			
Penicillin Calcium (845 U./Mg.)	0.06%	1 mo.	60	89
Lactose (dried)	43.5%	2 mo.	38	82
Triacetin (dried)	q. s.	5 mo.	..	22

(Cont'd on p. 374)

TABLE IV.—STABILITY OF OINTMENTS OF PENICILLIN IN OTHER WATER-MISCIBLE BASES

(*Cont'd from p. 373*)

Formula		Time	Per Cent of Theoretical Penicillin Potency: Room Temp.	Refrig. Temp.
Penicillin Calcium (845 U./Mg.)	0.056%	0	78	
Calcium Gluconate (dried)	46.5%	2 mo.	24	30
Triacetin (dried)	*q. s.*	5 mo.	16	21
Penicillin Calcium (845 U./Mg.)	0.06%	0	96	
Corn Starch (dried)	52.0%	2 mo.	56	85
Propylene Glycol Distearate	5.0%	5 mo.	40	84
Triacetin (dried)	*q. s.*	8 mo.	8	78
		15 mo.	..	51
Penicillin Sod. Cryst. (1620 U./Mg.)	0.03%	0	78	
Corn Starch (dried)	52.0%	3 mo.	35	77
Propylene Glycol Distearate	5.0%	7 mo.	28	69
Triacetin (dried)	*q. s.*			
Penicillin Pot. Cryst. (1583 U./Mg.)	0.03%	0	63	
Corn Starch (dried)	52.0%	3 mo.	59	62
Propylene Glycol Distearate	5.0%	6 mo.	31	62
Triacetin (dried)	*q. s.*			

Availability of Penicillin in Various Ointment Bases.—It was desirable to determine the readiness with which penicillin diffused from various ointment bases into the surrounding medium, especially those containing petrolatum in which it was found to be most stable. Consequently bacteriological tests were carried out by placing about 1 Gm. of the ointment being tested on a yeast extract agar plate or cup inoculated with *Staphylococcus aureus*. The zone of inhibition was measured after twenty-four hours at 37°. Table V gives a few examples of the results obtained from these tests.

TABLE V.—AVAILABILITY OF PENICILLIN IN VARIOUS OINTMENT BASES

Ointment	Potency, U./Gm.	Radius of Inhibition Zone on Plate in Mm.	Radius of Inhibition Zone on Cup in Mm.
Calcium Penicillin in Cottonseed Oil (5%) and Petrolatum Base	400	8	8
Calcium Penicillin in Mineral Oil and Petrolatum Base	440	7	8
Calcium Penicillin in Cottonseed Oil (40%) and Petrolatum Base	225	8	10
Calcium Penicillin in Wool Fat, Mineral Oil and Petrolatum Base	150	5	8
Sodium Penicillin in Cottonseed Oil (5%) and Petrolatum Base	825	8	11

It was concluded from the above results that penicillin in a base composed chiefly of petrolatum diffuses from the base into the surrounding aqueous medium. Similar behavior would be expected when the ointment is applied to wounds or to the eye. Other workers (9, 10) have used different means to measure the availability of the penicillin in similar ointment bases and have come to the same conclusions.

CONCLUSIONS

1. Impure sodium penicillin (400 u./mg.) is very unstable in an aqueous or a nonaqueous water-miscible ointment base. It has only limited stability in an anhydrous petrolatum base.

2. Calcium penicillin (500–650 u./mg.) is much more stable than the impure sodium salt in the same or similar bases.

3. The crystalline sodium or potassium salts of penicillin of high potency (1583–1620 u./mg.) are equal, if not superior, to the impure calcium salt for ointment purposes as far as stability is concerned.

4. Penicillin ointments are somewhat more stable at refrigerator temperature than at higher temperatures.

5. The presence of sulfadiazine, sulfathiazole, benzocaine, or epinephrine in a penicillin ointment does not appreciably influence the stability of the penicillin, while water, Carbowax, zinc stearate, and dried aluminum hydroxide gel hasten its decomposition to a marked degree.

REFERENCES

(1) Coomber, R. B., *Arch. Dermatol. and Syphilol.*, **52**, 246(1945).
(2) Leffingwell, G., and Lesser, M. A., *Med. Record*, **159**, 291(1946).
(3) Dahl, R. J., THIS JOURNAL, *Pract. Pharm. Ed.*, **6**, 120(1945).
(4) Greey, P., and Hebb, H. D., *Can. Med. Assoc. J.*, **52**, 550(1945).
(5) Neuroth, M. L., Lee, C. O., Christian, J. E., and Jenkins, G. L., THIS JOURNAL, **35**, 321(1946).
(6) Gots, J. S., and Glazer, A. M., *War Med.*, **7**, 168 (1945).
(7) Spencer, J., Bishop, E. J., and Ricks, A., *Lancet*, **250**, 127(1946).
(8) Templeton, H. J., Clifton, C. E., and Seeberg, V. P., *Arch. Dermatol. and Syphilol.*, **51**, 205(1945).
(9) Macek, T. J., Gakenheimer, W. C., and Daughenbaugh, P. J., *J. Investigative Dermatol.*, **8**, 209(1947).
(10) Clymer, H. A., and Ferlauto, R. J., THIS JOURNAL, **36**, 211(1947).

The Effect of Adrenochrome upon Experimental Glycosuria in the Rat*

D. J. INGLE, D. A. SHEPHERD, and WILLIAM J. HAINES†

Methods for the preparation of adrenochrome and its oxime are described. The effects upon glycosuria of both substances when administered to male rats having pancreatic diabetes and similar rats having alloxan diabetes are reported. The action of adrenochrome under different controlled feeding conditions was determined. This action was demonstrable, but not great. Adrenochrome oxime was without effect under the conditions of the several experiments described.

ADRENOCHROME is an oxidation product of epinephrine. Its preparation was first described by Green and Richter (1). Kisch (2) has reviewed studies of its biological properties. It has been reported that adrenochrome has been used successfully in the treatment of clinical diabetes (3, 4). Snyder, Leva, and Oberst (5) found that adrenochrome and iodoadrenochrome were ineffective in lowering the blood glucose level of normal rabbits, potentiating the effect of insulin, or neutralizing the action of epinephrine.

In the present studies on rats having alloxan diabetes and pancreatic diabetes it was found that large doses of adrenochrome caused a small decrease in the level of glycosuria in force-fed animals and that in alloxan-diabetic rats which ate *ad libitum* there was a more striking decrease in the level of urinary glucose associated with a decrease in voluntary food intake.

METHODS

Adrenochrome.—Adrenochrome was prepared by the method of Buchnea as described by McCarthy (4, 6). In typical preparations 4.58 Gm. (0.025 mole) of epinephrine, was dissolved in a mixture consisting of 125 ml. of absolute methanol and 2.25–2.50 ml. of formic acid, sp. gr. 1.20. To the solution was added 17.33 Gm. (0.075 mole) of silver oxide, and the mixture was stirred mechanically for 50–120 seconds. During this period the reaction mixture was kept at 38–40° by periodic application of an ice bath. At the end of the reaction the silver and silver oxide were removed quickly from the mixture by suction filtration and were washed with 15–25 ml. of fresh absolute methanol. The combined filtrates were cooled in an ice bath for fifteen to thirty minutes. The red crystalline precipitate of adrenochrome was collected by filtration, washed with a little cold absolute methanol and dried on the filter for fifteen minutes under a tube filled with Drierite. The yields obtained in this procedure varied from 0.84 Gm. (18.8%) to 1.28 Gm. (28.6%) depending on the conditions, the highest yield of 28.6% being obtained with 2.5 ml. of formic acid, a reaction time of sixty seconds and a crystallization period of fifteen minutes. Samples of adrenochrome prepared in this manner melted with decomposition, the decomposition point varying with the manner of heating the sample. Decomposition points obtained by dropping small amounts of material on a preheated Fisher-Johns melting-point apparatus were from 132° to 136°. The spectra, in the region 220–600 mμ, of representative samples of adrenochrome in absolute methanol were identical, within limits of experimental error, and, resembling the curve found by Ellis (7) for a cruder product, had maxima at 299 mμ (k = 57) and 479 mμ (k = 25.5). The blood-pressure response in an anesthetized, atropinized dog to an intravenous injection of a typical preparation of adrenochrome was approximately equivalent to only 0.03 μg. of epinephrine per mg. of adrenochrome. A sample for analysis was dried first at reduced pressure over Drierite and finally at 10μ Hg over phosphorus pentoxide at room temperature.

Anal. Calcd. for $C_9H_9O_3N$: C, 60.4; H, 5.06; N, 7.82. Found: C, 59.57, 59.65; H, 4.94, 4.64; N (Kjeldahl), 7.32, 7.37.

Adrenochrome is very soluble in water and methanol and is insoluble in benzene and the less-polar organic solvents. In the presence of moisture it decomposes to a black, water-insoluble, melanin-like substance. In the solid crystalline state some samples were stable for months, as evidenced by the similarity of their spectra and that of freshly prepared adrenochrome, while other samples, presumably somewhat moist, decomposed rapidly.

Adrenochrome Monoxime.—In the preparation of adrenochrome monoxime 1.00 Gm. (5.6 millimole) of adrenochrome was added, with shaking, to a solution of 1.00 Gm. of hydroxylamine hydrochloride and 2.00 Gm. of sodium acetate in 25 ml. of water. The mixture was shaken briefly and allowed to stand for approximately one hour. The bright orange precipitate was collected and twice recrystallized quickly from water, the first time at 0° and the second at room temperature. The final product, dried for thirty minutes on the filter and amounting to 0.40 Gm. (31%), was adrenochrome monoxime dihydrate, m. p. 178–182° dec. after changing from orange to yellow at 97°. Values for the m. p. reported in the literature are: monohydrate, 192° dec. (8); sesquihydrate, 278° (1).

Anal. Calcd. for $C_9H_{10}O_3N_2{:}2H_2O$: C, 46.94; H, 6.13; N, 12.17. Found: C, 46.82, 47.17; H, 5.66, 5.90; N (Dumas), 12.20, 11.94. Elementary analyses and color changes of samples given drying treatment beyond that described above indicated that the anhydrous material was unstable. The

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ultraviolet absorption spectrum of a methanol solution of the dihydrate displayed a fairly sharp maximum at 325 mμ ($k = 65 \pm 7$) and a broad, rather indefinite one at 390–405 mμ ($k = 25$–30). The position of the latter and the exact shape of the spectral curve may depend on the age of the sample.

Feeding Regimen.—Male rats of the Sprague-Dawley strain were maintained on Purina Dog Chow until they reached a weight of 310 Gm. One series of animals was depancreatized by the method of Ingle and Griffith (9). The second series of animals was made diabetic by injecting alloxan intraperitoneally in doses of 25 mg. every other day until glycosuria occurred. In most of the animals 2 doses of alloxan were sufficient to cause some degree of diabetes, but more resistant animals were given as many as 6 injections. After diabetes was established all of the animals were placed in metabolism cages and were fed a medium carbohydrate diet made according to Table I. Some of the animals were force-fed by stomach tube each morning (8:30 to 9:15) and late afternoon (4:15 to 5:00). The techniques and diet were modifications of those described by Reinecke, Ball, and Samuels (10). During the period of adaptation to forced feeding, the amount of diet was gradually increased to prevent the development of "food shock." The animals were brought to a full feeding of 26 cc. (29 Gm.) of diet per rat per day on the fifth day. The food for *ad libitum* eating was placed fresh each morning in clean, glass jars. The amounts of food consumed each day were determined by weighing at the same time each morning. There was no wasting of this fluid diet by the animals.

The animals were kept in an air-conditioned room with temperature at 74 to 78° F. and humidity at 30% to 35% of saturation. Twenty-four-hour samples of urine were collected at the same hour (8:00–8:30 A.M.) and were preserved with thymol. Urine glucose was determined by the method of Benedict (11).

The adrenochrome was made up in water solution and was given by subcutaneous injection either once or twice daily in a volume of 1.0 cc. per injection. The adrenochrome oxime was made up in sesame oil and was given once daily in a volume of 1.0 cc.

EXPERIMENTS AND RESULTS

Experiment 1.—Six rats having alloxan diabetes were treated with 5 mg. of adrenochrome daily for seven days. Five of the 6 rats showed a temporary small decrease in the level of glycosuria, but by the end of seven days the average amount of glucose excreted was as great as during the preinjection period (approximately 4 Gm. per rat per day). These data are not summarized in the charts. During this experiment it was found that adrenochrome is unstable in water solution and that even the powder frequently undergoes some change over a period of several days. It was considered that the failure of the compound to have a more significant and prolonged effect upon the level of urinary glucose may have been due to its instability.

Experiment 2.—Five rats which had alloxan diabetes were treated with 5 mg. of adrenochrome oxime daily for seven days. Five additional rats were treated with sufficient insulin (2 to 12 units daily of crystalline Zinc Insulin, Lilly) to control partially the glycosuria. These animals also received 5 mg. of adrenochrome oxime daily for seven days. In none of these animals was there evidence that adrenochrome oxime modified the level of glycosuria. Larger doses of the compound killed the animals. This compound was relatively stable in powder form. These data are not summarized in the charts.

TABLE I.—MEDIUM CARBOHYDRATE DIET

Constituent	
Cellu flour (Chicago Dietetic Supply)	120 Gm.
Osborne & Mendel salt mixture	40 Gm.
Diet yeast (Pabst)	100 Gm.
Wheat germ oil	10 Gm.
Cod-liver oil	10 Gm.
Vitamin K (2-methyl-1,4-naphthoquinone)	100 mg.
Mazola oil	200 Gm.
Casein (Labco)	160 Gm.
Starch	200 Gm.
Dextrin	190 Gm.
Sucrose	200 Gm.
Water to make total of	2000 cc.

Experiment 3.—Adrenochrome was prepared twice each week and the water solution was prepared a few minutes before it was injected into the animal. Six rats having alloxan diabetes were treated with 5 mg. of adrenochrome daily for ten days. All of the rats showed some decrease in glycosuria during the administration of adrenochrome. During the preinjection period the average amount of urinary glucose per rat per day was 2994 mg. It fell to an average of 1770 mg. during the injection period, and increased to an average of 2374 mg. during the postinjection period. These data are not summarized in the charts.

Experiment 4.—Six rats having alloxan diabetes and 6 rats having pancreatic diabetes were treated with 10 mg. of adrenochrome daily (divided doses) for seven days. The adrenochrome was prepared twice each week and was made up in water solution a few minutes before each injection period. All of the rats having alloxan diabetes and 5 of the rats having pancreatic diabetes showed a decrease in glycosuria during the injection period. The average amounts of glucose excreted rose to preinjection levels when the injections of adrenochrome were stopped. These data are summarized in Fig. 1.

Experiment 5.—Twelve rats having alloxan diabetes were studied during *ad libitum* eating. Six rats were treated with 5 mg. of adrenochrome daily for seven days and 6 rats were treated with 10 mg. daily for seven days. The adrenochrome was prepared twice each week and was made up in water solution a few minutes before each injection period. The amounts of food eaten were accurately measured. During the administration of the 5-mg. dose of adrenochrome 4 of the 6 rats showed a significant decrease in food intake and 5 of the animals showed a decrease in glycosuria. During the administration of 10 mg. of adrenochrome daily all of the animals showed a decrease in food intake and a decrease in glycosuria. When the injections were stopped these changes were reversed. These data are summarized in Fig. 2.

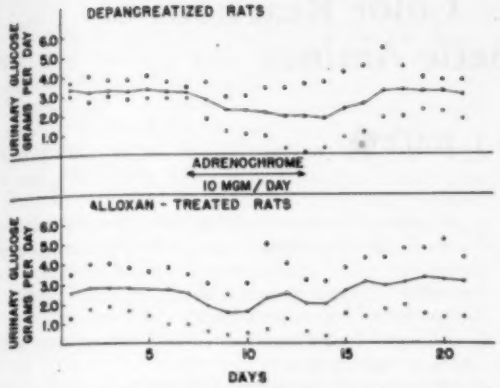

Fig. 1.—The effect of adrenochrome in force-fed, diabetic rats. Averages and range of individual values for glycosuria. Six rats in each of the two groups.

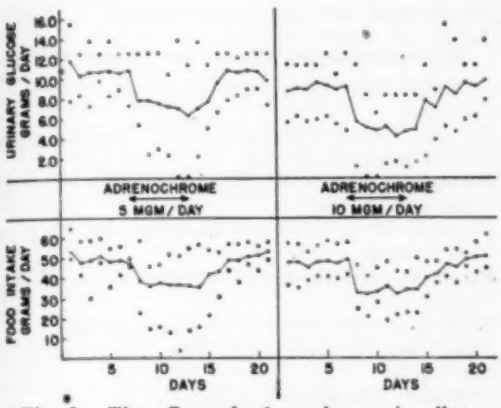

Fig. 2.—The effect of adrenochrome in alloxan-diabetic rats eating *ad libitum*. Averages and range of individual values for glycosuria. Six rats in each of the two groups.

DISCUSSION

It was shown in these experiments that the administration of adrenochrome to diabetic rats can cause a striking decrease in glycosuria, which is secondary to a decrease in voluntary food intake. In addition, there was a small but consistent average decrease in the level of urinary glucose when the intake of food was kept constant by forced feeding. These changes were not striking, but their consistent occurrence under rigid conditions of experiment probably represents a significant response to large doses of adrenochrome.

There have been many reports on compounds and crude natural products having an insulin-like action but in no case has acceptable supporting evidence been offered. It has not been shown that the effect of adrenochrome is related to the action of insulin. Snyder, Leva, and Oberst (5) found adrenochrome to be ineffective in lowering the blood glucose of rabbits. Insulin has a very striking effect upon the glucose load tolerated by the eviscerated rat (9, 12) but we have tested adrenochrome under identical conditions with negative results. Adrenochrome is a toxic compound. It is possible that its small effect upon experimental diabetes is related to its toxicity and is entirely unspecific. Ingle (13) found that injections of 4% formaldehyde caused a decrease in the glycosuria of the force-fed, partially depancreatized rat. The whole problem of the effect of various classes of toxins and of other forms of stress upon diabetes remains to be explored in the force-fed animal.

SUMMARY

Adrenochrome and adrenochrome oxime were tested for an effect upon glycosuria in male rats having pancreatic diabetes and in similar rats having alloxan diabetes. Doses of 5 and 10 mg. of adrenochrome per day caused a small decrease in the level of glycosuria in the force-fed rats. When the glycosuria was partially controlled by insulin the effect of adrenochrome remained slight. The administration of adrenochrome to diabetic rats which ate *ad libitum* caused a more striking decrease in the level of urinary glucose, which was associated with a decrease in food intake. Adrenochrome oxime was without any demonstrable effect in these experiments.

The preparation and some properties of adrenochrome and adrenochrome monoxime are described.

REFERENCES

(1) Green, D. E., and Richter, D., *Biochem. J.*, **31,** 596 (1937).
(2) Kisch, B., *Exptl. Med. Surg.*, **5,** 166(1947).
(3) Greiff, C., and Stocklein, J., *Med. Klin.*, **39,** 603 (1943).
(4) McCarthy, C. L., Report No. 47, issued by the Office of the Publication Board, Department of Commerce, 1945.
(5) Snyder, F. A., Leva, E., and Oberst, F. W., THIS JOURNAL, **36,** 253(1947).
(6) McCarthy, C. L., *Chemie & Industrie*, **55,** 435(1946); *Chem. Abs.*, **40,** 7180(1946).
(7) Ellis, S., *J. Pharmacol. Exptl. Therap.*, **79,** 364(1943).
(8) Veer, W. L. C., *Rec. trav. chim.*, **61,** 638, 646(1942).
(9) Ingle, D. J., and Griffith, J. Q., "The Rat in Laboratory Investigation," J. B. Lippincott Co., Philadelphia, 1942, Chapter 16.
(10) Reinecke, R. M., Ball, H. A., and Samuels, L. T., *Proc. Soc. Exptl. Biol. Med.*, **41,** 44(1939).
(11) Benedict, S. R., *J. Am. Med. Assoc.*, **57,** 1193(1911).
(12) Ingle, D. J., Nezamis, J. E., and Prestrud, M. C., *Am. J. Physiol.* **149,** 489(1947).
(13) Ingle, D. J., *Endocrinology*, **29,** 838(1941).

Microtoxicology. I. Color Reactions of Sympathomimetic Amines*

By THOMAS J. HALEY†

Color reactions by which fifteen sympathomimetic amines may be differentiated and classified are described. These color reactions, in combination with polariscope readings, should enable the toxicologist to identify not only any one of the fifteen sympathomimetic amines but also the isomer present.

THE PREVALENT use of sympathomimetic amines in inhalers, nose drops, tablets, and other forms of medication has led to abuses that are of toxicological importance. Recently there have been two reports dealing with desoxyephedrine toxicity (1, 2). Amphetamine also has caused similar toxic manifestations (3–7).

Means for the identification of the exact amine causing the clinical toxicity have not been adequately developed. It has been shown that the heavy metal alkaloidal precipitants offer a rapid means for differentiating these potent substances (8). Earlier investigators were, in general, interested in the preparation of derivatives of the pure compounds and worked with much larger quantities than are usually available to the toxicologist. In this manner amphetamine has been identified as the benzoyl derivative (9) and *d*-desoxyephedrine as the picrolonate (10).

Inasmuch as the opium and other alkaloids have been identified by their color reactions with various reagents it was decided that a study of the color reactions of fifteen sympathomimetic amines with these reagents should be undertaken.

EXPERIMENTAL

The following alkaloidal reagents were prepared according to the directions in Autenrieth and Bauer (11): Marquis, Frödhes, Rosenthaler-Turk, Mandelin and Meckes. Sanchez reagent (12) was also prepared. To several drops of each reagent on a spot plate or 1–2 cc. of the reagent in a small test tube was added about 1 mg. of the following sympathomimetic amines: *dl*- and *l*-ephedrine; *d*-, *dl*-, and *l*-desoxyephedrine; *d*- and *dl*-amphetamine; Paredrine; Nethamine; Vonedrine; *d*-, *dl*-, and *l*-Neosynephrine; *dl*-Synephrine and Tuamine. The following results were obtained.

Marquis Reagent (*Formalin-Sulfuric Acid*)

dl-Ephedrine, *l*-Ephedrine } Orange, dark brown, and finally wine colored.

d-, *dl*-, and *l*-Desoxyephedrine, *d*- and *dl*-Amphetamine, Vonedrine } Orange-red, orange-brown, and finally dark brown.

Paredrine—Purple-red and finally dark purple.

Nethamine—Yellow and finally dark brown.

d-, *dl*-, and *l*-Neosynephrine—Rose, red-orange, and finally dark brown.

Synephrine and Tuamine—Colorless.

Frödhes Reagent (*Molybdic Acid*)

dl-Desoxyephedrine—Weak yellow.

Paredrine—Emerald-blue with a yellow ring.

d-, *dl*-, and *l*-Neosynephrine—Powder blue.

All the other compounds gave colorless solutions.

Mandelin Reagent (*Vanadium-Sulfuric Acid*)

dl- and *l*-Ephedrine—Reddish brown, reddish on stirring.

d-, *dl*-, *l*-Desoxyephedrine—Red, emerald-green, deep green on stirring.

Vonedrine—Yellowish green, emerald-green, deep green on stirring.

d- and *dl*-Amphetamine—Olive-green, greyish green on stirring.

Paredrine—reddish brown, blackish green on stirring.

Nethamine—Blood-red, no change on stirring.

d-, *dl*-, and *l*-Neosynephrine, Synephrine } Black, deep green on stirring.

Tuamine—Black, no change on stirring.

Rosenthaler-Turk Reagent (*Arsenous Acid*)

All the compounds were colorless before heating, and after both heating and the addition of concentrated hydrochloric acid a faint yellowish tinge was noted.

Meckes Reagent (*Selenous Acid*)

d-, *dl*-, *l*-Desoxyephedrine, Vonedrine, Nethamine } Lemon yellow

Paredrine—Brick red.

d-, *dl*-, and *l*-Neosynephrine—Reddish purple.

Synephrine—Lavender.

Sanchez Reagent (*Alcoholic Dimethylaminobenzaldebyde-Sulfuric Acid*)

dl- and *l*-Ephedrine, *d*-, *dl*-, and *l*-, Desoxyephedrine, Vonedrine, Tuamine } Emerald-green residue, pale green solution.

d-, *dl*-Amphetamine—Yellowish green residue, yellowish green solution.

Paredrine—Reddish brown residue, yellowish brown solution.

d-, *dl*-, and *l*- Neosynephrine—Orange residue, pale orange solution.

Synephrine—Yellowish brown residue, deep yellow solution.

* Received Oct. 9, 1947, from the Department of Pharmacology and Toxicology, University of Southern California, School of Medicine, Los Angeles, Cal.

† Fellow in Pharmacology and Toxicology.

DISCUSSION

Marquis reagent enables the investigator to differentiate definitely these amines from other alkaloids because their color reactions are distinctly different (13). However, desoxyephedrine, amphetamine, and Vonedrine react as a group and cannot be distinguished from one another by this test. This is not surprising when one considers their close structural relationship, amphetamine (beta-phenylisopropylamine), desoxyephedrine (beta-phenylisopropylmethylamine), and Vonedrine (beta-phenyl-n-propylamine). The compounds most easily distinguished by this test are ephedrine, Nethamine, Paredrine, and the Neosynephrines. All of these give distinctly different color reactions which allow them to be differentiated from one another and the other amines tested. However, this test as well as the other tests employed will not distinguish between the various optical isomers.

Frödhes reagent enables one to identify Paredrine and the Neosynephrines. Both codeine and narcotine give color reactions similar to Paredrine but the presence of the yellow ring with the latter is sufficient to differentiate it from them. The color response of papaverine is similar to that of the Neosynephrines but the former finally becomes yellow whereas the latter retains their blue color.

The nonreactivity of these amines with Rosenthaler-Turk's reagent enables the toxicologist definitely to distinguish them from the opium alkaloids which give definite color reactions with this reagent.

Mandelin's reagent gives distinct color reactions with all of these amines but here again it is impossible to distinguish between optical isomers. This reagent enables the toxicologist to distinguish between such structurally related compounds as amphetamine and desoxyephedrine, which with other reagents give the same color reaction. The colors produced by the amines are sufficiently different from those recorded in the literature (13) to enable them to be distinguished from such alkaloids as papaverine, colchicine, and morphine.

Meckes' reagent gives readily distinguishable color reactions with the Neosynephrines, Paredrine, and Synephrine, which are sufficiently different from those given by other alkaloids to enable them to be identified. The other amines tested gave reactions closely approximating those given by colchicine and veratrine.

Sanchez's reagent is of little value for the differentiation of these amines because of the number of them that give the same color reaction. However, it could be used for the identification of Paredrine, the Neosynephrines, and Synephrine.

When the reactions of the substances tested with these six reagents are considered as a whole, one must conclude that they present a rapid method for classification even though, with the possible exception of Paredrine, individual compounds or their isomers cannot be differentiated. However, by the process of elimination it would be possible to narrow the group down to one or two specific substances. Because these compounds contain an asymmetric carbon atom, the toxicologist could positively identify not only the compound but its exact isomer through its specific rotation.

SUMMARY

A method for the differentiation and classification of fifteen sympathomimetic amines using six common toxicological reagents has been presented. Paredrine and the Neosynephrines give the best color reactions of all the compounds tested. The color reactions reported, in combination with polariscope readings, would enable the toxicologist to identify rapidly not only the toxic agent but also the exact isomer causing the toxic symptoms.

REFERENCES

(1) Carratala, R., and Calzetta, J. C., *Semana med. (Buenos Aires)*, **1943**, II, 985; through *Chem. Abstr.*, **38**, 1031(1944).
(2) Dittmar, F., *Deut. med. Wochschr.*, **68**, 266(1942).
(3) Waud, S. P., *J. Am. Med. Assoc.*, **110**, 206–7(1938).
(4) Apfelberg, B., *ibid.*, **110**, 575–6(1938).
(5) Smith, L. C., *ibid.*, **113**, 1022–3(1939).
(6) Trevan, J. W., *Proc. Roy. Soc. Med.*, **32**, 291(1939).
(7) Hertzog, A. J., Karlstrom, A. E., and Bechtel, M. J., *J. Am. Med. Assoc.*, **121**, 256–7(1943).
(8) Haley, T. J., THIS JOURNAL, **36**, 301(1947).
(9) New and Non-Official Remedies, 1946, American Medical Association, Chicago, Ill., p. 279.
(10) Dultz, G., *Z. anal. Chem.*, **120**, 84(1940).
(11) Autenrieth, W., and Bauer, K. H., "Die auffindung der gifte und stark wirkenden arzneistoffe," 6th Auflage, 1943, Theodor Steinkopff, Dresden and Leipzig; J. W. Edwards, Ann Arbor, Mich., 1944, pp. 330–4.
(12) Sanchez, *Semana med. (Buenos Aires)*, **1936**, 425; through Merck Index, ed. 5, 1940, p. 897.
(13) Autenrieth, W., and Bauer, K. H., *op. cit.*, pp. 148–9.

The Preparation of Tri(Hydroxymethyl)Sulfanilamidomethane*

By GAIL A. WIESE† and JAMES W. JONES‡

Tri (hydroxymethyl) sulfanilamidomethane was prepared by treating tri(hydroxymethyl)-aminomethane with *p*-acetoaminobenzenesulfonyl chloride in an aqueous medium with controlled temperature and subsequent hydrolysis of the acetyl compound. Proof of the presence of three hydroxyl groups in the molecule was accomplished by acetylation and subsequent saponification. The compound was tested *in vitro* against two Gram-negative and two Gram-positive organisms, using sulfathiazole sodium as a standard. In no case was its bacteriostatic action equal to that of sulfathiazole sodium.

THE OBJECT of this investigation was to prepare a "sulfa" drug which would be far more soluble than those now in use without destroying the therapeutic activity inherent to this class of drugs.

In reviewing the literature, it was found a large series of acylamidobenzenesulfonalkanolamides and aminobenzenesulfonalkanolamides were prepared by Adams, Long, and Johanson (1). They established the N^1 alkanol derivatives to be more soluble than their acyl derivatives. Crossley, Northey, and Hultquist (2) reported the preparation of a series of N^1 substituted aliphatic sulfonamides, including several N^1 hydroxyalkyl derivatives. They prepared 2-methyl-2-sulfanilamido-1-propanol and 2-methyl-2-sulfanilamido-1,3-propanol. It was noticed they did not report the preparation of a third compound in this immediate series, namely, tri(hydroxymethyl)sulfanilamidomethane.

The preparation of tri(hydroxymethyl)sulfanilamidomethane was attempted by three different methods: First, by reacting *p*-acetamidobenzenesulfonyl chloride with tri(hydroxymethyl)aminomethane in an excess of dry pyridine, as described by Goldfarb (3). This reaction progressed smoothly, but difficulty in separating the sulfonamide from the pyridine hydrochloride prohibited the isolation of tri(hydroxymethyl)sulfanilamidomethane. The second attempted synthesis was by the method described by Crossley, Northey, and Hultquist (4), using sodium hydroxide as the condensing agent. As in the previous method, the difficulty of separating the sulfonamide from the reaction mixture prohibited isolation of the compound. The third method, and the one used to obtain the sulfonamide in a pure form, was the method described by Adams, Long, and Johanson (1), involving the use of an additional molecular equivalent of the alkanolamine to act as the condensing agent.

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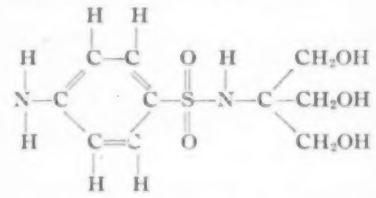
† Graduate student and Fellow in The American Foundation for Pharmaceutical Education.
‡ Professor of pharmacy, State University of Iowa, College of Pharmacy, Iowa City, Iowa.

EXPERIMENTAL

Tri(hydroxymethyl)sulfanilamidomethane was prepared by a modified method used by Adams, Long, and Johanson (1). Since the acetyl derivative did not separate from the reaction mixture, it was hydrolyzed within the mixture in the presence of hydrochloric acid. The mixture was then evaporated to dryness under reduced pressure. The mass remaining was broken up with a glass rod, 100 cc. of absolute alcohol added, and the mixture again heated on the water bath until all except the alkanolamine hydrochloride was dissolved. The mixture was cooled, the alkanolamine hydrochloride filtered off and washed with cold absolute alcohol. The filtrate was again cooled and dry ammonia gas bubbled into the alcoholic liquid until a precipitate of ammonium chloride separated which was removed by filtration. The alcoholic filtrate was decolorized with charcoal, then evaporated to about one-fourth its original volume on the water bath. On cooling, the liquid became syrupy in consistency, and on standing in the refrigerator overnight, a precipitate of tri(hydroxymethyl)sulfanilamidomethane formed. The precipitate was collected on the filter and washed with cold 50 % alcohol. The yield of crude product was 60–65% of the calculated. The precipitate was recrystallized once from 50% alcohol and twice from 95% alcohol. On the final crystallization, prisms were obtained which melted at 159.5–161°.

Anal.—Calcd. for $C_{10}H_{16}N_2SO_5$: N, 10.14. Found: N, 10.15.

Tri(hydroxymethyl)sulfanilamidomethane may be represented by the following formula:



One Gm. of tri(hydroxymethyl)sulfanilamidomethane is soluble in 90 cc. of water or 110 cc. of alcohol at room temperature, 25 cc. of hot water and in 20 cc. of hot alcohol. On cooling to room temperature the sulfa derivative did not separate from hot water or hot alcohol. Treatment of tri(hydroxy-

methyl)sulfanilamidomethane with acetic anhydride and glacial acetic acid gave a penta-acetyl derivative which melted at 193–195°.

Tri(hydroxymethyl)sulfanilamidomethane was tested bacteriostatically, *in vitro*, against four microorganisms, *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus hemolyticus*. A simple qualitative method was used, in which three tubes, each containing 5 cc. of beef infusion, were inoculated with one of the organisms named above. To one of the tubes 25 mg. of tri(hydroxymethyl)-sulfanilamidomethane was added, to another 25 mg. of sulfathiazole sodium, the third tube being used as a control. Tri(hydroxymethyl)sulfanilamidomethane showed inhibition of growth on *Escherichia coli*, *Proteus vulgaris*, and *Streptococcus hemolyticus*, but in no case was the inhibition comparable to that of sulfathiazole sodium. It failed to inhibit the growth of *Staphylococcus aureus*, as did sulfathiazole sodium.

SUMMARY

A new derivative of sulfanilamide was prepared and tested *in vitro* against two Gram-negative and two Gram-positive organisms.

1. Tri(hydroxymethyl)sulfanilamidomethane is more soluble than sulfanilamide, sulfathiazole, and sulfadiazine in either alcohol or water.

2. Tri(hydroxymethyl)sulfanilamidomethane was tested *in vitro* on four microorganisms and found to be less bacteriostatic than sulfathiazole.

REFERENCES

(1) Adams, R., Long, P. H., and Johanson, A. J., *J. Am. Chem. Soc.*, **61**, 2342(1939).
(2) Crossley, M. L., Northey, E. H., and Hultquist, M. E., *ibid.*, **62**, 532(1940).
(3) Goldfarb, A. R., *ibid.*, **67**, 1352(1945).
(4) Crossley, M. L., Northey, E. H., and Hultquist, M. E., *ibid.*, **60**, 2217(1938).

Book Reviews

Vitamins and Hormones, Volume V. Edited by ROBERT S. HARRIS and V. THIMANN. Academic Press, Inc., New York, 1947. xvi + 478 pp. 15 x 23 cm. Price $7.50.

The ever-increasing number of papers on the biological, chemical, and clinical properties of the vitamins and the hormones makes the wisdom of an annual review in these fields apparent.

In Volume V of these reviews, the authors have been carefully selected, as always, to condense and evaluate the most important recent developments.

The recent synthesis of vitamin A makes a review of such synthetic methods advisable. This is ably done by Nicholas A. Milas of Massachusetts Institute of Technology. The question of the physiological availability of the vitamins is reviewed by Doctors Melnick and Oser. These authors also evaluate the limitations of nonbiological methods for determining the vitamins. Other reviews in the vitamin field deal with thiamine and neurophysiology, the effects of pterylglutamates in man, the vitamin requirements of the chick, and the requirements of the mouse.

In the hormone field most of the reviews are of pharmaceutical importance. The reviews consider the properties of the growth and adrenocorticotropic hormones, the effect of exogenous estrogens on the male mammal, the biology of antithyroid agents, the use of androgens in women, and the clinical uses of testosterone in the male.

A valuable feature of the volume is the cumulative index to the first five volumes.

The Aseptic Treatment of Wounds. By CARL W. WALTER, A.B., M.D., assistant professor of surgery at Harvard University, and senior associate in surgery at the Peter Bent Brigham Hospital in Boston. The Macmillan Company, New York. 1947 ix + 372 pp. 21 x 28.5 cm. Price $9.00.

Beginning with a history of the development of the concept of asepsis, and discussing in full detail the various phases of aseptic technique, this book offers a ready reference for surgeons, nurses, pharmacists, and bacteriologists. Other hospital personnel concerned with the necessary space and equipment for carrying out aseptic technique in the hospital will also find this volume valuable. A long list of references and a good index add to its usefulness.

Of particular interest to pharmacists in hospitals is the chapter on the preparation of parenteral fluids in which the author outlines specific procedures for the preparation of solutions. The design of the still, source of water supply, equipment necessary, pyrogen test, cleaning of glassware, and sterilization of solutions are all discussed in detail and diagrammatic illustrations of stills, apparatus, etc., are included. Illustrations of the autoclave along with information on its operation are covered in a separate chapter.

The importance of aseptic technique and how it is accomplished are emphasized throughout this volume, giving special attention to all aspects of the destruction of bacteria in connection with instruments, dressings, and the skin. Routines for carrying out aseptic procedures in the operating room and central supply are outlined in detail along with drawings to illustrate each step.

Air-borne contamination, blood and plasma facilities, control of communicable disease, and maintenance of sterilizing equipment are also covered.

Growth of Plants. By WILLIAM CROCKER. Reinhold Publishing Corporation, New York, N. Y., 1948. xii + 459 pp. 16 x 23 cm. Price, $10.

The title of this volume does not adequately cover the contribution that the author has made. The book is actually an extensive research review of the many practical problems dealing with the physiology of plants as investigated by the staff of the famous Boyce Thompson Institute for Plant Research. Among more outstanding discussions are those dealing with the physiological effects on plants of many gases, factors affecting dormancy in seeds and buds, a discussion of plant hormones, and the report of the effects on plants of an extensive number of controlled environmental conditions including such factors as artificial light, low temperatures, spectral glass, radiation, and ultraviolet light. Discussions are well illustrated and graphed.

In so far as the book is a critical summary of only those researches carried on at Boyce Thompson Institute it does not cover any one field exhaustively. For example, there is much more general information to be desired than what is covered under research on insecticides, fungicides, plant hormones, and many others. A generous literature citation, however, somewhat makes up for this.

The author stimulates an interest in a variety of plant research problems the results of which are applicable not only to horticulture but also to the public health.—H. W. YOUNGKEN, JR.

Detoxication Mechanisms; The Metabolism of Drugs and Allied Organic Compounds. By R. TECWYN WILLIAMS. John Wiley and Sons, Inc., New York, 1947. viii + 288 pp. 13.5 x 21.5 cm. Price $5.50.

During more recent years pharmacologists have placed greater emphasis on the action of drugs on animals and man than on the action of animal tissues on drugs. Around the turn of the century considerably more research took place which had as its goal knowledge of the metabolism (detoxication) of drugs and organic chemicals in the animal body.

In addition to oxidation and reduction of drugs, animal tissues can bring about certain synthetic and conjugation reactions. The author describes in detail the ability of tissues to form glucuronides, ethereal sulfates, glycine, cysteine, ornithine and glutamine conjugates, and to methylate and acetylate certain substances. Also covered is the ability of animals to convert the highly toxic cyanides into the less toxic thiocyanates.

Compounds covered are the most important aliphatic compounds; aromatic hydrocarbons; phenols; aromatic alcohols, ethers, aldehydes, ketones amides and acids; organic cyanides; aromatic nitro, amino and azo compounds; sulfones, sulfonic acids and sulfonamides; terpenes and camphors; heterocyclic compounds; and organic arsenicals.

This book is a helpful review largely because it shows the great gaps in our knowledge of the metabolism of drugs.

Approaches to Tumor Chemotherapy. Edited by FORREST RAY MOULTON. American Association for the Advancement of Science, Washington, 1947. viii + 442 pp. 18.5 x 26 cm. Price $7.75.

Human cancer is one of the most complex and baffling diseases known to man.

Although many forms of cancer are known to exist they all behave biochemically substantially alike.

The biochemical similarity of cancers coupled with the fact that a few leads have been developed for their chemotherapeutic "cure" offers a new hope for finding a genuine cure or prophylaxis. The papers in *Approaches to Tumor Chemotherapy* were presented at the 1945 Gibson Island Research Conference on Tumor Chemotherapy or were related to the field but presented at the 1946 Conference.

This book is divided into six parts, the first of which deals with a historical introduction. From the five papers comprising Part 2, it is apparent that the field has come a long way in developing a methodology. This is important since any studies become hit-or-miss unless systematic biological methods are developed.

Part 3 deals with the nutritional approach. This approach has proved to be very fruitful in many other fields and it is reasonable to expect that it will be helpful in the tumor field also. Among other subjects considered in this section is the effect of folic acid treatment on various types of tumors.

Part 4 emphasizes the influence of bacterial products on tumors and especially deals with certain polysaccharides which have proved to have the ability to cause the regression of some tumors.

Part 5 consists of seven papers on the nitrogen mustards and Part 6 contains six clinical papers dealing with urethane and oil-soluble organo-metallic compounds, among other things.

The reader will close this book with the realization that tumor chemotherapy poses many more questions than it answers. For the first time, however, there appear points of departure which may lead to spectacular developments in the future.

ERRATUM

In the review of the book entitled *Modern Cosmeticology*, by Ralph G. Harry, which was published in the August issue of THIS JOURNAL, page 338, the price was incorrectly stated as $10.00. The correct price of this book is $12.00.